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STUDIES ON ARMILLARIA MELLEA (VAHL) QUEL., INFECTION, PARASITISM, AND HOST RESISTANCE [1]

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INTRODUCTION

Armillaria mellea has long been recognized as an important plant parasite, mainly attacking forest and fruit trees and causing in them a serious root rot. It grows luxuriantly as a saprophyte on the dead roots and stumps of trees and, according to Kusano (*25*),[2] may also act in a mycorrhizal relationship.

A great deal of effort has been expended upon control measures in many parts of the world, but with little success. Much has been said about resistant rootstocks, but practically nothing is known regarding the nature of resistance to this fungus. The present work had as its object a study of the mode of entrance, the resistance of various rootstocks, and the nature of such resistance. It was believed that more was to be gained by examining a considerable number of different hosts than by confining the investigations to a more exhaustive study of one susceptible and one resistant host. The principal part of the study dealt with a histological and cytological investigation of the mode of infection of susceptible and resistant hosts. The term "infection" as here used means either the penetration of the fungus into the host without further development, or penetration with subsequent production of a diseased condition. Further work was undertaken on the nature of resistance.

Not much definite knowledge exists as to susceptible and nonsusceptible roots or tubers. In this study those rootstocks or plants which usually escape destruction in "*Armillaria* spots" (soil areas where the disease occurs) will be considered as resistant, and those usually destroyed as susceptible.

REVIEW OF LITERATURE

The method of invasion of tree roots by *Armillaria*, whether through wounds or through the healthy bark, received some attention from the early German workers. Hartig (*14*, *15*) in 1873 and 1874 was the first to establish the fact that *Armillaria mellea* (then called *Agaricus melleus*) was the cause of a serious root disease,

[1] Received for publication July 26, 1933; issued April 1934. Contribution from the Division of Plant Pathology, University of California, Berkeley, Calif.

[2] Reference is made by number (italic) to Literature Cited, p. 216.

which had been known for some time but which was believed to be due to an excess of resin. He further proved the connection between the rhizomorphs and the fruiting bodies. At that time he was unable to determine whether the fungus entered through a sound root of the attacked conifer or through a wound. He observed, however, that when trees were close together none escaped the disease. At a later date he (*16*) concluded that the rhizomorphs penetrate the sound roots of conifers and that wounds in the roots are not necessary to allow entrance; he gave no details of the fungal invasion. He was uncertain as to the entrance of the fungus into plum and cherry but considered it a parasite on them. In 1894, after experimenting with oaks in which he cut the top off and allowed the roots to sprout, Hartig (*17*) concluded that *Armillaria* is a wound parasite and is unable to enter uninjured roots, that if the tissue is in an active growing state the fungus does not invade it, and that only resting tissues are attacked. Later work (*18*), however, caused him to modify his view. When healthy oaks had a single root removed and left for 2 years before being examined, it was found that the cuts were covered with rhizomorphs, that the fungus had penetrated for a depth of 3 mm, and was there held in check with no further penetration into the wood or cortex. From this he concluded that the fungus was not a parasite on a healthy oak, not even a wound parasite.

Brefeld (*6*) developed methods for growing this fungus in pure culture and used these cultures to infect thick, freshly dug roots of the Scotch pine, on which he stated the fungus most often occurred parasitically. He dug out and brought the roots "uninjured and fresh" into direct contact with the tips of the rhizomorphs. Penetration occurred at once, and the fungus emerged at the cut end between the bark and wood after 5 to 7 days. The act of penetration was said to require 1 to 2 days, but no description or illustration of the exact process is given. It is stated that the rhizomorph as it creeps over the surface of the root forms lateral branches which penetrate directly into the root; or the tip itself may enter. Hiley (*21*) and Zeller (*46*) do not believe this method to be capable of testing the normal rhizomorphic invasion into healthy, uninjured roots. They believe it is impossible to remove roots from the soil without some undetectable surface injury and do not consider roots to be in a healthy, living condition when so treated. Evidence obtained in the examination of Austrian forests is presented by Cieslar (*10*) who concluded from his field observations that entrance by *Armillaria* into the roots of oak, ash, and elm is gained through wounds in the crown or the roots below it. The fungus sometimes found in irregularities on the surface of oak and elm roots was always walled off by a periderm layer before it penetrated to the cambium. He notes the importance of insect injuries as a means of gaining entrance. De Bary (*4*) in 1884 stated that the strands penetrate into the healthy living cortex of roots of healthy trees, especially the conifers, but he probably accepted Hartig's views.

It thus appears that the early German workers were fairly well agreed that the fungus can enter the sound living bark of conifers but can only enter the broadleaf trees through some wound in the bark. They give none of the details of actual penetration nor do

they state how the fungus enters the sound cortex. Brefeld makes the statement that branches of the rhizomorph form on the lower side and penetrate directly but does not state how the entrance takes place.

Neger (*32*) in 1908 very briefly described the penetration of the rhizomorph into the roots of the silver fir. He found, in trees displaying a dying-back of the lower branches, a network of rhizomorphs surrounding the taproot and in some places entering the bark. At such points an appresorium or suction plate had formed, from which the penetrating rhizomorph branch grew. The latter first enters the outer dead portion of the bark and later pushes through the cork into the tissue below. New and deeper layers of cork then form; but Neger observed that these are often in turn penetrated by the rhizomorph.

In 1911 Kusano (*25*) investigated the relation of *Armillaria mellea* to a Japanese orchid, *Gastrodia elata*, to which the fungus acts in a mycorrhizal connection. He states that the rhizomorph grows on the surface of the tuber, fastens itself to it, and sends infection branches into the tissues of the tuber. In so doing the fungus first dissolves the outermost suberized cork cells and then by dissolution of the dividing walls attacks the underlying living cells forming a "lysigenic space for the advance of the growing infection strand." The single hyphae which composed the infecting rhizomorph then enter the surrounding cells as single mycelial threads and no longer maintain the rhizomorphic structure. The symbiosis is established. Kusano further observed that the fungus may at times attack the *Gastrodia* tuber parasitically and in so doing causes compression and a brownish discoloration of the cells surrounding the infecting strand. This is not apparent in the symbiotic relation. During the investigation Kusano also found the fungus attacking potato tubers parasitically. He concludes that the action on the potato is much the same in its general aspect as the parasitic action on the *Gastrodia* tuber. The work of Kusano applies to such parenchyma tissue as that of a tuber.

It cannot be assumed, without further proof, that entrance into the hard tissue of a root takes place in exactly the same manner. Horne (*22*) has studied the general aspects of the *Armillaria* problem, probably more thoroughly than any other American worker, but he has dealt with the subject mainly from a practical point of view and not from the standpoint of detailed microscopical study. As to penetration he suggests that—

> when the tip of the rhizomorph comes to a healthy root the very small microscopical threads of which it is composed, seem to loosen like the cut end of a rope and the individual threads penetrate into the bark and start a new infection.

It would be assumed from this that the author believes penetration is through the sound healthy bark. Nechleba (*31*), observing the behavior of *A. mellea* in forest trees, considers wounds or a decrease in the vigor of the host essential to entrance of the fungus. Barss (*3*) believes that rhizomorphs may penetrate into healthy bark but more often gain entrance through injured roots or crowns. Hiley (*21, p. 159*) studied the *Armillaria* root rot of the larch under

forest conditions in England. He makes some rather sweeping statements regarding the penetration of fungi into tree roots.

> Though the early pathologists seem commonly to have accepted the theory that fungi pierce the sound bark of trees, no authenticated instance of this has ever been recorded, and the trend of recent opinion has been more and more in the direction of admitting the possibility of infection only by wounds or by outflanking the bark protection.

Hiley further states: "The mode of infection employed by *Armillaria mellea* has never been critically examined * * *" and considers the problem a difficult one to elucidate. He maintains that with rhizomorphic infection it is "* * * still a question whether entrance is effected (i) through healthy, uninjured bark, (ii) through wounds, or (iii) through dead roots", and that his observations support the view that rhizomorphs can enter only through damaged or dead roots. Samofal (*36*) concluded after 20 years' observations in the pine forests in the Provinces of Chernigof and Kief in Ukraine that *A. mellea* seems to be a parasite and saprophyte on conifers and only a saprophyte on broadleaf trees, gaining entrance to the latter when the roots have been injured.

Zeller (*46*) in 1926 concluded that in rhizomorphic infection, Horne's idea of penetration in which the single threads of the rhizomorph strand enter individually "comes the nearest to describing actual infection." He observed that healthy roots during their growth sometimes come into very close contact with diseased roots and that the healthy roots become diseased at the point of contact. Without following the actual process he postulates that toxic substances produced by the growth of the fungus in the diseased root, upon coming in contact with the healthy bark, enter through the lenticels, causing a shallow disorganized spot in the tissue which blisters and later flakes with the development of cork under it. The process is repeated until several layers of flakes form and the fungus finally passes into the cambium. He thinks this action resembles a type of "burning" which may occur when organic debris is brought into close contact with young bark. He believes that further evidence of the toxic substances produced by this decay is seen in the effect on the top of the tree.

Another theory on penetration advanced by Zeller deals with the entrance of the vegetative mycelium of *Armillaria* at the point of emergence of lateral roots, through the rupture in the bark parenchyma made by the root in forcing through. From the theories presented by this author it is evident that he considers a wound or other disturbance necessary before *Armillaria* can enter. The method of entrance by contact of diseased and healthy roots is alluded to in the writings of other authors, among whom are Lawrence (*26*), Barrett (*2*), Birmingham (*5*), Hendrickson (*20*), and Samofal (*36*); and the phenomenon has undoubtedly been observed by many others who make no mention of it. Old roots or root pieces may live for many years in the soil and remain as a source of infection by this method.

While the experimental work reported on later in this paper was in progress an article by Day (*12*) appeared, which did not come to the attention of the author until this work was completed. In this article Day describes and illustrates the mode of entrance employed by the rhizomorph in the infection of conifers and discusses their

susceptibility to attack. He examined and studied naturally infected roots taken from the forest and in so doing recognized the possibility that "it would perhaps have been better had it been collected from such trees specially inoculated with, or exposed to, attack by a pure culture of the fungus." Day concluded that the attack of *Armillaria mellea* is solely by rhizomorphs. Attachment of this organ to the host is quite apart from penetration and occurs when the rhizomorph grows into the dead, outer cork cells. The fungus appears to exert a toxic influence upon the host tissue under the rhizomorph as a preliminary to penetration. The rhizomorphs penetrate the cork by dissolution instead of by mechanical rupture. A toxic influence precedes the advance of the rhizomorph. Secondary cork layers form to prevent entry of the parasite. This process continues in some instances and invagination results. The evidence indicates that *A. mellea* is able to penetrate an uninjured and apparently healthy host. Observations in the forest led this author to the opinion that variations in susceptibility to attack among species of conifers do not coincide with their susceptibility to death after attack and is possibly accounted for by external environmental factors. Day has undoubtedly contributed more than any other worker to the solution of the problem of the method by which *A. mellea* enters its hosts. His work is, however, concerned only with coniferous roots.

Rayner (*35*) using a pure culture of *Armillaria mellea* failed to obtain satisfactory infection in inoculated seedling Corsican pine and Douglas fir grown aseptically in sand. Under the conditions of the experiment the fungus did not organize rhizomorphs, and it is stated only small cankers, subsequently exfoliated, were produced by the action of single mycelial threads. Such cankers on roots were practically confined to the neighborhood of emerging laterals.

From this review it is seen that there still exists some dispute concerning the details of infection, at least in woody plants and trees; that no critical microscopical studies have been reported regarding the mode of entrance into tree roots under controlled conditions; that no systematic attempt to investigate and compare penetration into the so-called resistant and nonresistant rootstocks has been reported; and that little or nothing is known regarding the nature of host resistance.

METHODS

The culture of *Armillaria mellea* used throughout this study was made from a root of an old prune tree (*Prunus domestica*) killed by the fungus. A culture was thus obtained from a strain known to be parasitic, which might not be the case if a single-spore culture had been used. The culture has been maintained on prune agar since isolation. Material used for soil inoculation was cultured on prunings from fruit trees autoclaved in battery jars with water, almost any kind of tree being satisfactory. A piece of plain glass was used to cover the jar. In this material the fungus grows rapidly and may fill an 8-inch jar in an interval of 2 months or less.

Tree seedlings to be tested were grown in a screened mixture of sand and greenhouse soil in frames set into the ground, the inoculum being placed from 5 to 6 inches below the surface of the soil. In order to avoid root injury to the seedling through transplanting, seed

was ordinarily used and was planted in the upper 2 inches. The peach pits were an exception; they had sprouted before planting, but the roots did not extend down more than 2 inches below the soil surface. This method eliminated, as far as possible, any root injury. Seedlings were dug for examination at various intervals, and occasionally one would show the stage desired. Persian (English) walnut (*Juglans regia*, Concord variety), northern California black walnut (*J. hindsii*), peach (*Prunus persica*), myrobalan (*P. cerasifera*), and French pear (*Pyrus communis*, Winter Nelis and Surprise varieties) comprised the list of seedlings used for study. All grew fairly well except the myrobalan, only a few of which came up and for some unknown reason these escaped infection. Myrobalans referred to later in this work were taken from another experiment in which 1-year-old seedlings were planted in 10-inch flowerpots for a resistance test and had grown there for 3 years. Carrots (*Daucus carota*), parsnips (*Pastinaca sativa*), dahlias (*Dahlia* sp.), and potatoes (*Solanum tuberosum*) were grown in boxes of inoculated soil much the same as the tree seedlings.

After the inoculum has been in the soil for several months, it will, on being dug and placed in a moist chamber, produce a large quantity of rhizomorphs in a period of 2 to 4 weeks (pl. 1, *B*), and if kept moist these rhizomorphs will attain considerable length. Objects like potato tubers may be brought into contact with the rhizomorphs and the invasion by the fungus watched. Such a method was used in some of the potato studies reported herein.

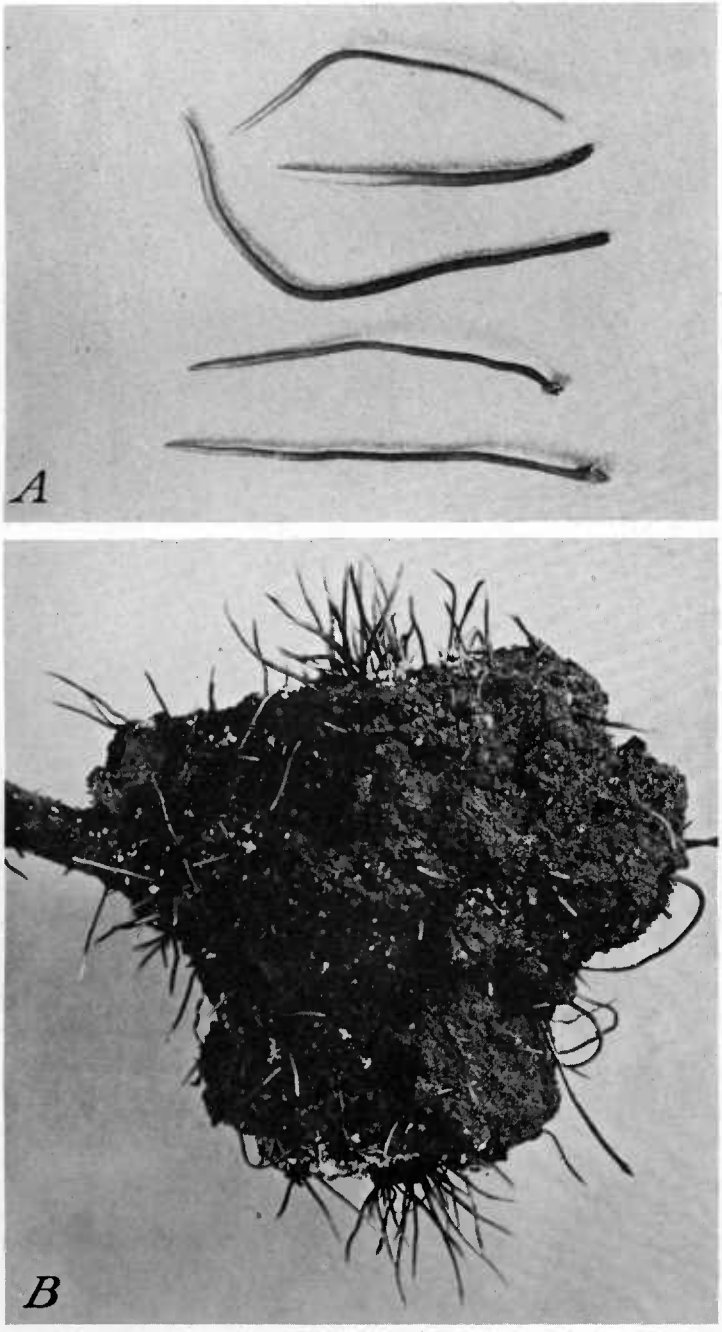
For killing and fixing agents, Rawlins' (*34*) alcohol-formalin-acetic formula and Flemming's weaker solution were used, and others tried. Flemming's weaker solution appeared to cause a little less shrinkage of the rhizomorph than did the others. Various stains were used during the study. Vaughan's (*40*) modification of Pianeze's 111b stain, and Flemming's triple were used most extensively. The former proved to be the best, giving good differentiation of the fungus, the host, and the diseased tissue. Both paraffin and free-hand sections were used throughout this study. With woody tissues the paraffin method is not ideal, the wood usually becoming too hard to section perfectly. The hydrofluoric acid treatment caused injury to the fungus and was not generally used. In order that the whole history of the lesion be known, its entire length was always sectioned. This proved definitely whether a wound or lenticel was originally present at the point of entrance.

Of the fleshy roots or tubers used for the infection study the potato, carrot, and parsnip were found to be susceptible, the potato being rather the most so. The dahlia tuber seemed to show more resistance but was not immune. Of the tree seedlings, field observations indicated that the Persian walnut and peach were very susceptible, the myrobalan had some resistance, while the French pear and black walnut were very resistant.

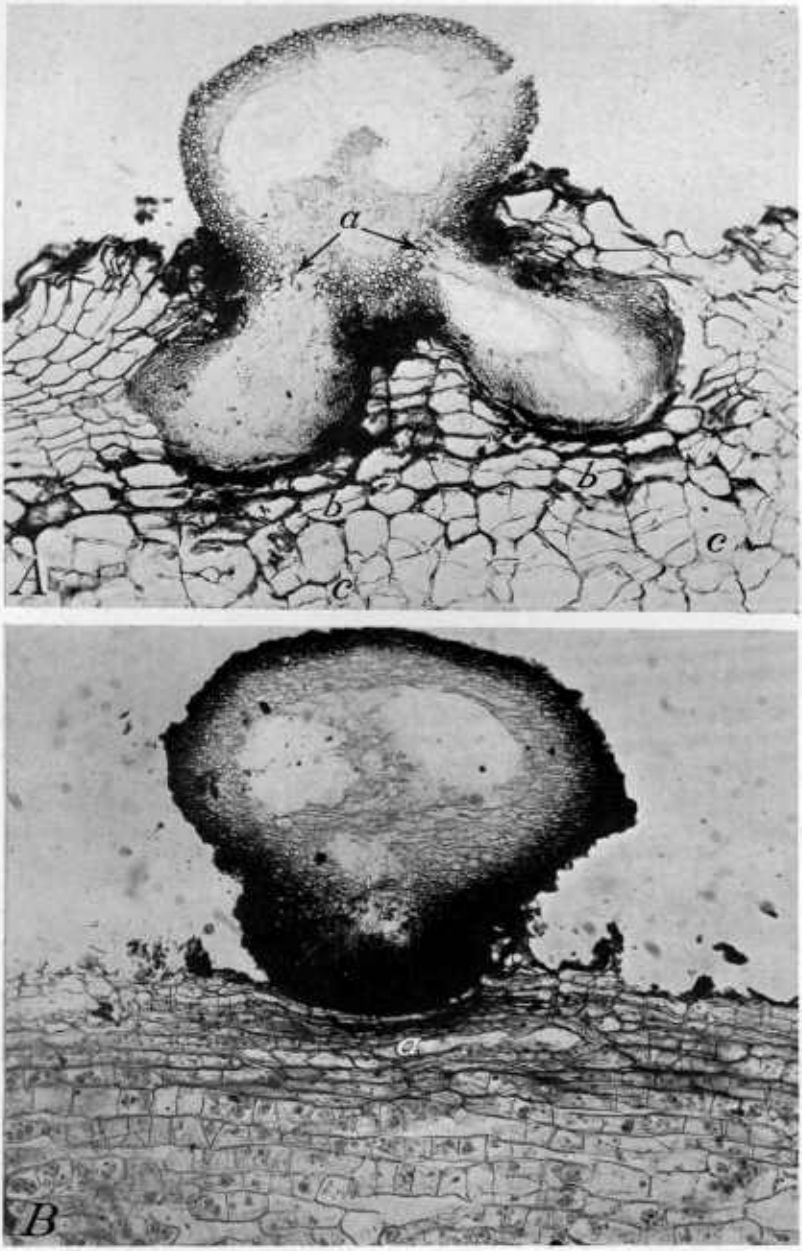
EXPERIMENTAL DATA

ATTACHMENT OF THE RHIZOMORPH

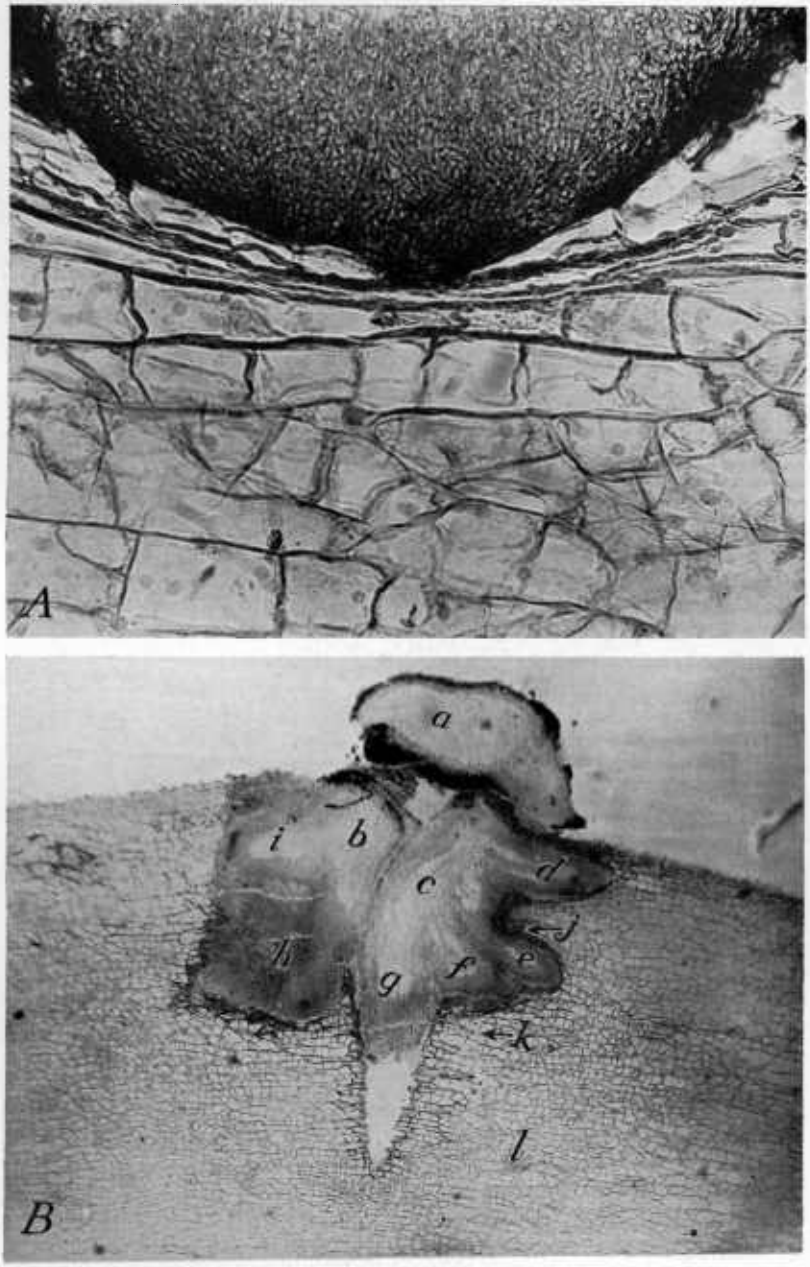
When the free end of the rhizomorph comes in contact with a root, it, in some cases, becomes rather firmly fixed. The attachment is brought about, partly at least, by the hardening of the mucilagi-



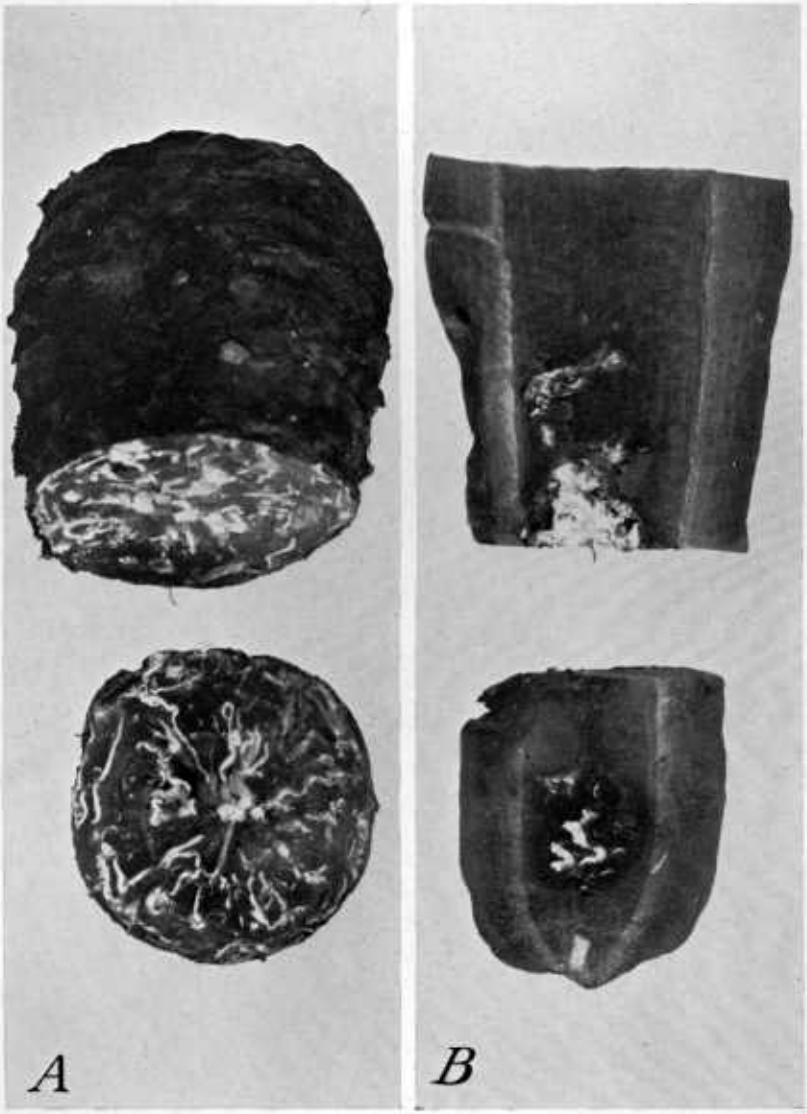
A, White tips of growing rhizomorphs. × 3. *B*, Rhizomorphs growing from a piece of inoculum in a moist chamber after having been in soil for 6 months. × 1.



A, Rhizomorph entering dahlia tuber: *a*, Remnants of crushed cells where branch rhizomorph originated; *b*, disorganized tissue; *c*, newly formed cork. × 90. *B*, Parsnip; first stages of entrance by a rhizomorph: *a*, Compressed and deeply staining tissue below invading rhizomorph. × 90.



A, Carrot; detail of the tip of an entering rhizomorph. × 240. *B*, Carrot; invading rhizomorphs beginning to branch: *a*, Parent rhizomorph; *b* and *c*, infection rhizomorphs; *d* to *i*, secondary branches; *j*, compressed and disorganized host tissue; *k*, affected but not disorganized tissue beyond *j*; *l*, normal tissue. × 22.



A, Carrot completely permeated by internal rhizomorphs. × 1. *B*, Rhizomorphs extending in pockets in the central cylinder of the carrot. × 1.

nous substance which envelops the rhizomorph close behind its white tip (pl. 1, *A* and *B*). This material, when dry, gives the shiny appearance to the surface of the rhizomorph, and when it dries in contact with the root surface helps in gluing the rhizomorph to the root. Single side hyphae developing from the rhizoromph tip and penetrating into the outer layer of cork cells act as anchors to hold the strand fast. In a moist chamber, on potatoes the rhizomorph has been observed to be attached rather firmly for an inch or more along the surface. Usually the attachment is not continuous, the rhizomorph being alternately loose and joined. The base of the white tip is sometimes firmly fastened and at other times free. This undoubtedly determines whether or not the rhizomorph is to become attached at that point, for after the mucilaginous substance has once hardened, there is no means by which the rhizomorph may become fastened.

DEVELOPMENT OF A BRANCH RHIZOMORPH

Branches normally develop at the point of contact of the attached rhizomorph and the root surface. They appear always to develop where the contact is very secure and never where the rhizomorph is unattached. They are to form the penetrating rhizomorphs (pl. 2, *A*). The branch originates in the inner cortical cells of the rhizomorph where some stimulus, thought by Brefeld (*6*) to be a contact stimulus, is set up causing the hyphae of the parent rhizomorph in this region to branch laterally. These hyphae then force their way through the outer cortical cells by tearing and crushing or otherwise destroying them and emerge as a branch. A few of the torn cell remnants often remain (pl. 2, *A*, *a*). Branches may be fairly numerous under an attached rhizomorph, apparently always developing on the side of the rhizomorph in contact with the host and seldom, if ever, from the opposite side. Their constant occurrence in the one position emphasizes the importance of the stimulus or stimuli initiating the process.

MANNER OF PENETRATION OF THE RHIZOMORPH

Fleshy Roots and Tubers

It has already been seen that the rhizomorph is apparently capable of attaching itself at any point on the root or tuber, and that branches form regularly where firm attachment has occurred. If infection now takes place, the branch must enter through the corky or suberized covering which protects the root or tuber. This must be done either by mechanical force, in which case the suberized cells would be either crushed or pushed aside, or by solvent action of the hyphae on the suberized layer with subsequent growth through the dissolved mass, or by the splitting apart of the cells which form the cork layer, at their middle lamellae, and entering in these splits. The manner of entrance into fleshy roots is similar in its general aspects for all those investigated. After entrance has once been gained, details of the effect on different roots vary slightly. The first stage of entrance is illustrated in plate 2, *B*, where the fungus is preparing to enter a parsnip. A detail at the tip of a rhizomorph is shown in plate 3, *A*, in which the initial stage of entrance into

a carrot is illustrated. It is readily seen that the rhizomorph enters as a unit, that the hyphae composing the penetrating rhizomorph tip are acting en masse, and that there are no single threads extending beyond this mass.

Up to this stage entrance is by mechanical force, the host cells under the rhizomorph being pushed in and slightly compressed. There is no destruction of tissue and very little evidence, if any, that there is any solvent action upon the suberized layer and certainly no splitting of these cells at the middle lamellae with penetration through the splits. But it will be noticed in plate 2, *B*, that the walls of the cells at *a* under the two outer layers of cork cells are stained very deeply, while similar cells not in this region do not take this heavy stain. This gives evidence that these cells are not normal and may be affected by something given off from the tip of the penetrating rhizomorph. The tip of the invading branch continues to push in, finally penetrating through the suberized layer into the tissue below. There is distinct evidence that mechanical pressure is playing a part, since some of the collapsed cells surrounding the tip are pushed to the side.

The formation of secondary branches by the penetrating rhizomorph often occurs at the next stage of growth in a fleshy root or tuber. Plate 3, *B*, *d* to *i*, illustrates such branching in a carrot. This particular section shows the parent rhizomorph (*a*) on the surface of the carrot but the branching from it of the infection rhizomorphs (*b* and *c*) does not show at their point of emergence, which is demonstrated in a later section where the two branches originate at about the same place on the parent, one from either side. In the section shown, after penetration has occurred, the branches (*b* and *c*) appear almost as one. Each shows the start of two or more branches which will soon develop, and from here the spread in a susceptible host is fairly rapid. After a young branch (pl. 3, *B*, *d* to *i*) has attained a little more extension it sends out from near its base single hypae which spread into the surrounding tissue and continue the destruction of the host cellular structure. As the tip of the branch extends, these side hyphae follow, growing out perpendicularly to its surface. In an advancing rhizomorph they were never found to extend ahead of the tip, but in one no longer extending they would probably be found growing from the tip. The cells immediately surrounding the secondary branches, as well as the primary invading branch, are affected chemically; their walls are closely compressed and the whole so changed that they now present only an indistinguishable mass, which with Pianeze's stain takes a dark-green color indicating death and partial destruction of the cells (pl. 3, *B*, *j*). For two, three, or more cells deep around this mass the stain is not taken normally (*k*). The walls may become a pale yellowish green if they assume any color at all. In some hosts they are slightly brown and fail to take the stain. The cell contents, if such are present, assume an even paler green. Beyond this (*l*) the cells appear normal, and both wall and contents take a pink stain. Observed macroscopically from the surface and in the fresh state, at the stage indicated in plate 3, *B*, or a little later, there is found under the attached rhizomorph a brown area, at first small and pale brown but soon enlarging and darkening at the center. It indicates that

the rhizomorph has entered and is acting parasitically on the host. Plate 4, *A*, illustrates a late stage in the destruction of the carrot.

Plate 2, *A*, illustrates a case soon after penetration has taken place in the dahlia, a somewhat resistant root. The branch of the rhizomorph has pushed through the cork layer intact down into the tissue under it and is affecting it chemically, as indicated by the different staining reaction at (*b*). Up to this point there has been no essential difference in detail between penetration into the susceptible or resistant roots, but now a difference is observable. Under the mass of affected cells in the resistant dahlia there has been formed by the host a layer of cork, which walls off from the remaining part of the tuber the disorganized area surrounding the entering rhizomorph, and further development of the latter appears stopped. In plate 2, *A*, this layer of cork (*c*) appears as a line of dividing cells, but when stained with Sudan III it gives the typical test for suberized walls. The development of this suberized layer around the infection branch is a rather constant response of this host to invasion by the fungus. Of 13 cases examined each showed a corky layer, or dividing cells preparatory to its formation, soon after entrance into the tuber had been effected. In the susceptible carrot and parsnip an imperfect line of dividing cells is occasionally found, but a definite layer of suberized cells is seldom formed. In the potato, the most susceptible of the fleshy roots or tubers studied, no case was found where dividing cells were forming. The significance of the cork layer will be discussed in a later paragraph.

Susceptible Woody Roots

The hardening of the woody tissue during dehydration makes it almost impossible to cut woody sections without some tearing by the knife, especially of the bast fibers and hard lignified walls. Since only the first stages of entrance were of importance at this time, in most instances the bark only was removed and sectioned, there being no need to study the wood below. The bark alone cut more easily than did the bark and wood together. The Persian walnut proved to be exceptionally good material for this study. It has a more fleshy root than the peach and cuts more easily.

The first stage of invasion is represented in plate 5, *A*, where a small infection branch (*a*) is penetrating through the cork tissue. The parent rhizomorph is not firmly attached to the host in this section, probably having been torn away during sectioning or previous handling.

A slightly later stage is illustrated in plate 5, *B*. The rhizomorph is expanding laterally in the cork tissue, destroying some of the suberized cells but here extending mainly by mechanical force. Chemical action is, however, not absent, as indicated by the disorganization of the tissue below the remaining 2 or 3 layers of cork cells at *a*. This section is through the approximate center of the entering rhizomorph and shows the deepest point to which the rhizomorph has penetrated. It gradually recedes as sections on either side are examined, leaving little doubt that the influence felt below the cork is ascribable to secretions of some sort from the rhizomorph diffusing through the remaining 2 or 3 layers of cork cells at *b*.

Plate 5, *A*, also shows considerable disorganization under the penetrating tip, but this is attributed, at least in part, to an adjoining deeper infection at a short distance from this one, with its influence extending out below the one illustrated. As in the case of fleshy roots, the infecting rhizomorph is penetrating as a unit with no indication of single hyphae radiating out in advance.

At the next step the rhizomorph pushes through the remaining cork and down into the tissue below, where branching usually takes place; its progress is essentially the same as in the fleshy roots previously described. In a young walnut seedling, as used in this study, the large tap root is very fleshy, being somewhat like a carrot in shape with a tapering shoulder at the top. The cortex is largely parenchyma tissue, enclosing a thin woody cylinder surrounding a large pith. The internal rhizomorphs of the fungus readily permeate the parenchyma of the cortex, quite the same as in the carrot or parsnip, and then grow through the thin woody cylinder into the pith where the growth is very rapid and may outstrip that in the cortex. The growth is so luxuriant that the pressure exerted by the rhizomorphs as they expand in the pith is sufficient at times to split the main root of the small seedling longitudinally.

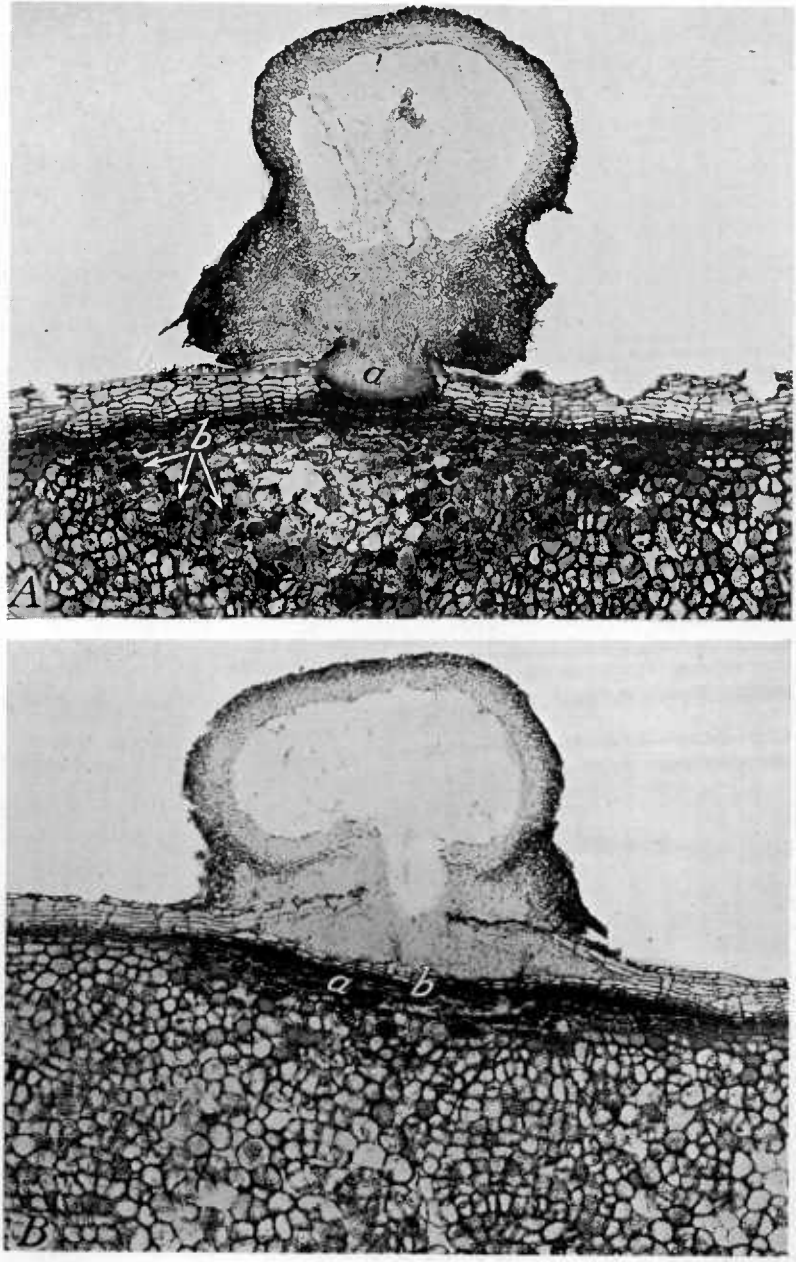
It was pointed out earlier that the rhizomorph may attach itself to the root for some distance and form infection branches at several points close together. This was well illustrated in a walnut root. A rhizomorph was attached rather firmly for a distance of 1¼ inches. Sectioning of this entire length of root revealed the fact that a branch of the rhizomorph had either entered or formed preparatory to entry at each of 15 separate points.

Of the many walnut roots examined in this study at least two were found in which entrance had been gained at a lenticel, but in the majority of cases entrance was through the apparently uninjured and unaffected cork layer of the root.

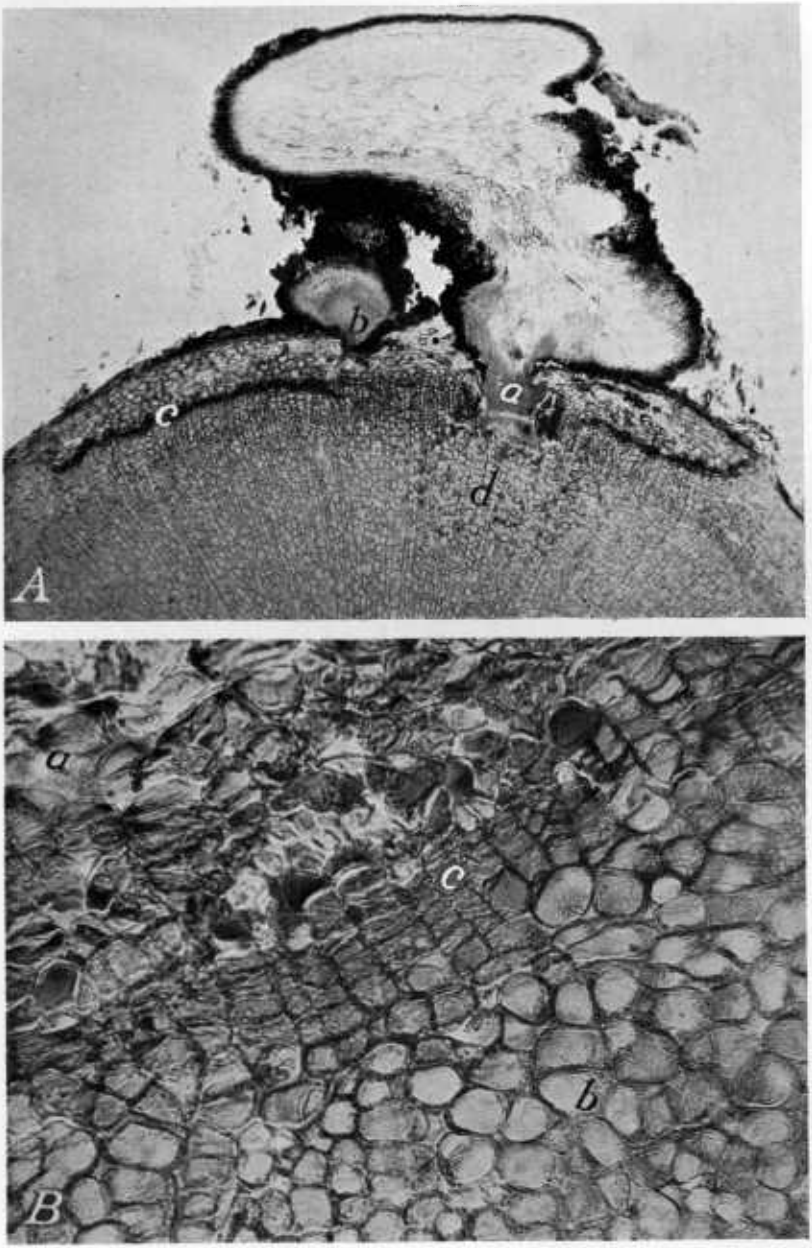
Although the roots of 20 or more seedling peaches were found infected at one or more places, it so happened that none was found at the time when actual penetration by the rhizomorph was taking place. Later stages of the infection, however, did not indicate that penetration was different in this root from those heretofore described. One feature of growth in the peach root which had not been noticed in any of those previously studied was the formation of cavities or pockets, filled with gum, in the roots or main stem, a short distance beyond the point where the internal rhizomorph was advancing. These cavities were always close to the cambium layer, sometimes just under it, at other times including the young wood and the cambium and a part of the phloem. They were always situated between the medullary rays, the latter seldom, if ever, being included in the cavity.

Resistant Woody Roots

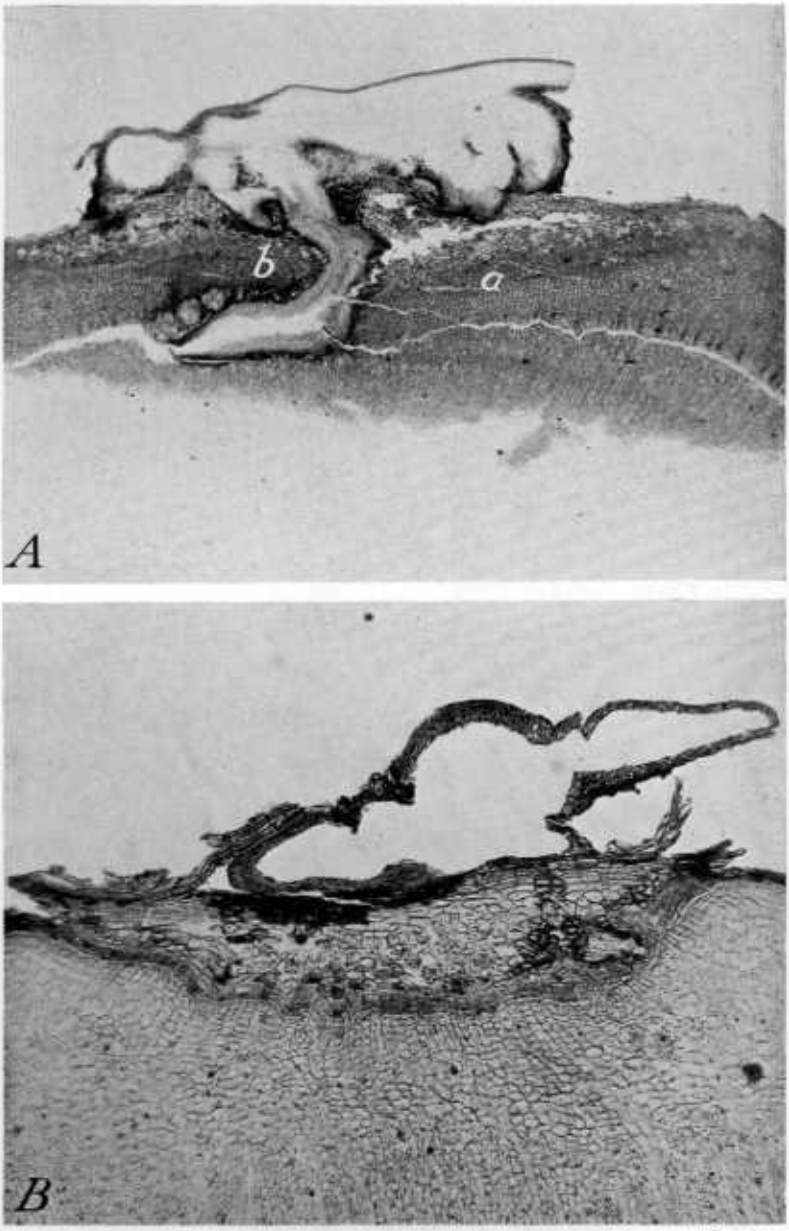
Seedlings grown from seed of the French pear, varieties Winter Nelis and Surprise, were used as resistant roots for this study. They were dug and examined from time to time over a period of 9 months to determine the mode of entrance of the fungus and the host reactions. The first stages of penetration into healthy pear tissue indicated that entrance was directly through the sound cork, just



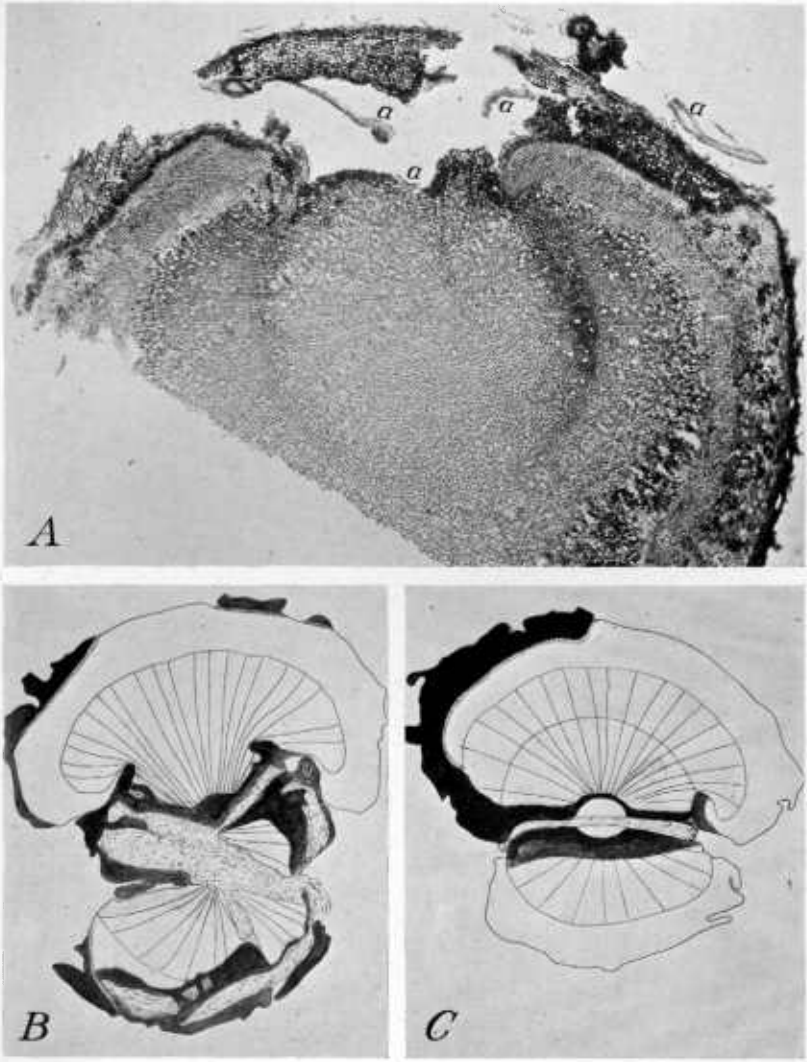
A, Persian walnut root (susceptible); first stages of penetration by a branch rhizomorph: *a*, Infection branch; *b*, deep staining deposit filling cells at edge of lesion. × 62. *B*, Persian walnut root; rhizomorph spreading laterally in the cork layer causing some compression and destruction of the cork cells: *a*, Disorganized tissue; *b*, a layer of cork 2 and 3 cells thick remaining between the rhizomorph branch and the disorganized tissue. × 62.



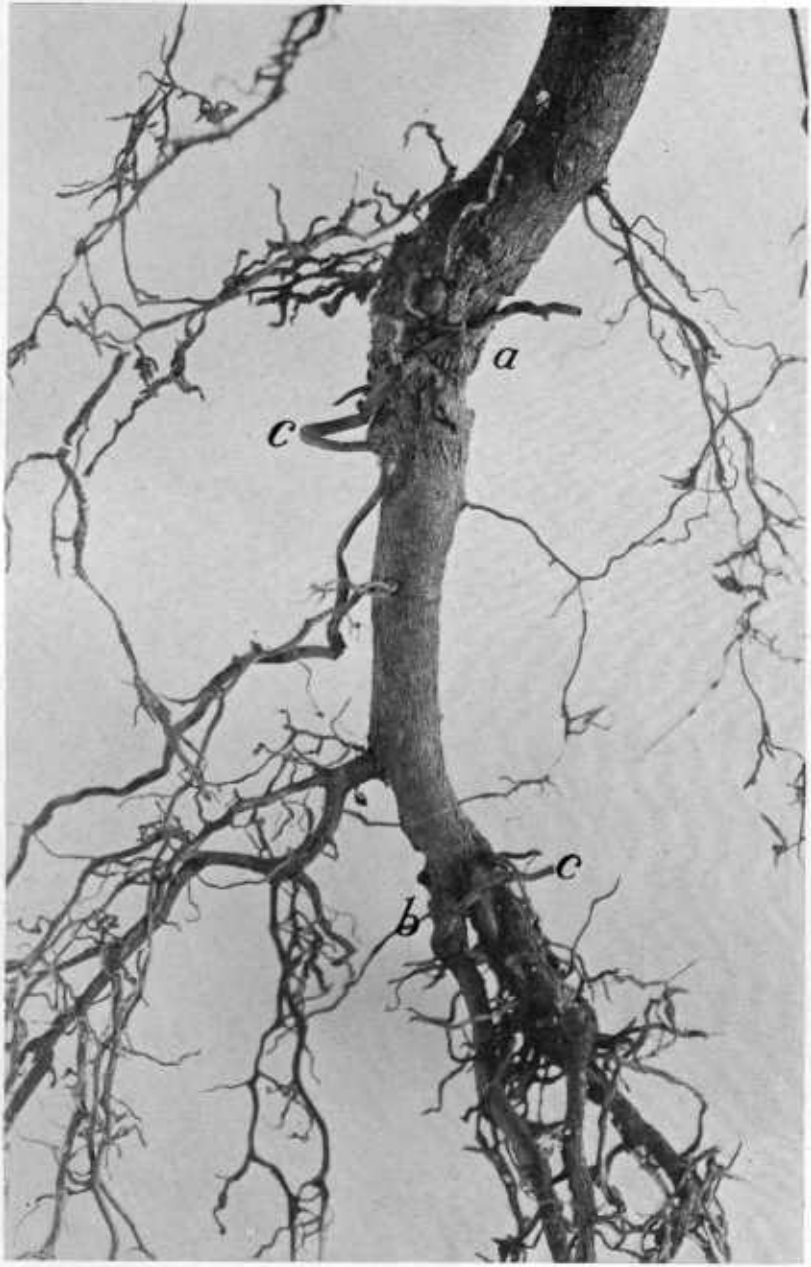
A, Pear root; entering rhizomorph: *a*, Extending below the cambium; *b*, a branch entering original cork layer; *c*, secondary periderm; *d*, affected wood. × 43. *B*, Pear root; detail of secondary periderm: *a*, Disorganized tissue inside lesion; *b*, normal tissue outside lesion; *c*, phellogen. × 230.



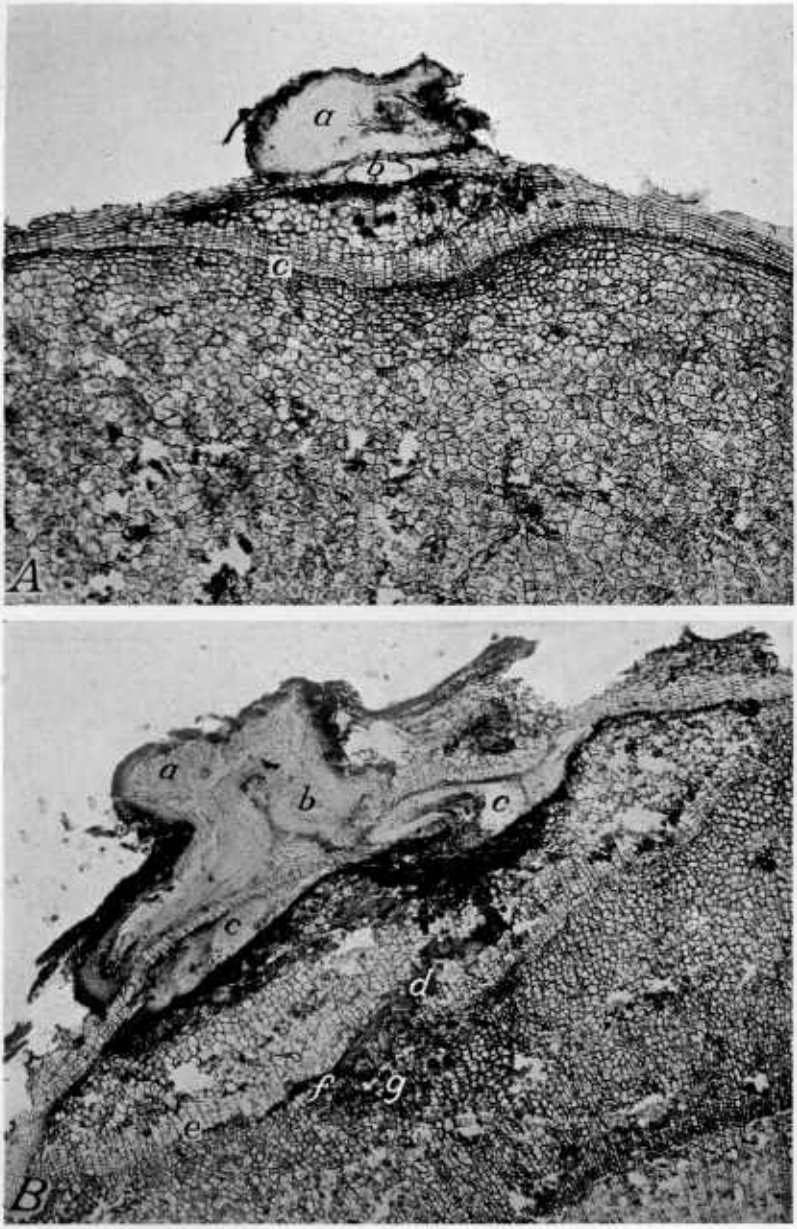
A, Pear root; rhizomorph entering wood below cambium: *a*, New cork; *b*, crushed cells. × 22. *B*, Pear root; rhizomorph failed to penetrate below the original cork; the wound has been walled off by a layer of secondary cork. × 62.



A, Pear root; an old deep lesion healing out: *a*, Remnants of old rhizomorph. × 24. *B*, Pear root; camera lucida drawing of a cross section of an affected tap root at approximate position where rhizomorph entered. *C*, Below *B* in the same lesion; crosshatching indicates the fungus; the very dark area the dead, browned wood or cortex; the lighter areas the less affected wood; and the uncolored areas the normal healthy tissue. × 11.



Pear root; *armillaria* lesions at *a* and *b*. The root is affected over its entire circumference at *a*; *c*, rhizomorphs. × 3.



A, Black walnut root; old rhizomorph on surface walled off by new cork: *a*, Old rhizomorph; *b*, branch rhizomorph which has penetrated original cork; *c*, new cork. × 38. *B*, Black walnut root; a rhizomorph with several branches, one of which is penetrating the secondary periderm: *a*, Original rhizomorph; *b*, primary branches; *c* and *d*, secondary branches; *e*, new periderm partially penetrated at point *f* by the branch rhizomorph *d*; *g*, disorganization in tissue at this point caused by rhizomorph branch *d* before completely penetrating through periderm *e*. × 28.

as in the susceptible walnut root. In plate 6, *A*, the rhizomorph has entered at *a* and is entering at *b*, but we are hardly justified in considering this latter to be entering sound tissue, for the entrance of the first rhizomorph has undoubtedly caused some disturbance in the cells under the second. When the invasion of the first rhizomorph took place some host reaction occurred, causing the root to start the formation of a periderm in the cortex tissue below. This shows as a dark line in plate 6, *A*, *c*. On the lesion side of this line, the cells are much disorganized (pl. 6, *B*, *a*), whereas on the other they appear quite normal (pl. 6, *B*, *b*), indicating that the influence from the rhizomorph, whatever it may be, has been checked at this line. It is plainly evident that the rhizomorph in plate 6, *A*, *a*, has not been checked in its advance by the formation of the periderm. It has broken through this layer and through the cambium and is causing some disorganization of the cells in the wood below, as evidenced by the deeper staining area at *d*. Another instance in which this secondary cork layer has been penetrated is illustrated in plate 7, *A*. The section has been badly broken in cutting, but the important features stand out clearly. The rhizomorph has broken through the young cork (*a*) and through the cambium and is growing in the wood. That some mechanical pressure is exerted is evidenced by the crushing of the cells above the rhizomorph in the region of the cambium at *b*, although this does not show clearly in the photomicrograph.

The rhizomorph does not in all instances break through the secondary cork layer in the manner illustrated in the foregoing cases. In plate 7, *B*, is shown an instance in which the rhizomorph failed to enter further than the primary cork layer. The lesion produced has been definitely walled off by the secondary periderm. This cork layer widens with the growth of the root, but any sloughing of its outer cells is prevented by the tissue forming the lesion, which remains intact on the surface. The loss of the outer cork cells surrounding the lesion finally leads to a situation where the old lesion is clinging to the surface of the perfectly normal appearing root. With the continuation of root growth it is finally lost.

In plate 8, *A*, a few remnants of an old rhizomorph (*a*) remain at the center of the lesion, enough to show the probable cause of the injury. The parasitism of the fungus has been overcome by the host and the latter is now attempting to heal the wound with growth from the cambium. Plate 8, *B*, illustrates a very severe lesion in the tap root of a small Surprise pear seedling. This section, made at about the place where entry occurred, shows the destruction which may be caused in the host and yet not kill the root. The internal rhizomorphs have split the root in half, almost completely killing one half and invading a part of the other, yet at this time the host has apparently overcome the fungus and is beginning to heal over the wound by cambial growth. Plate 8, *C*, illustrates a section lower down in the same lesion where the root is split but less affected on the two sides. The fungus is making no headway other than the splitting of the root, and healing is progressing. Many similar instances of this general effect were observed, especially in later examinations after the early infection had ceased to be active. A surface view of such a lesion is shown in plate 9.

From data presented thus far it will be seen that resistance in the pear can hardly be accounted for by the failure of the rhizomorph to enter the host. Nor does it appear that any morphological obstructions are present and responsible for resistance. In this host the fungus readily enters and may penetrate to the cambium and into the wood below, at times killing tissue deep in an individual root. It might be expected that the fungus would now be free to penetrate any portion of the root. A wound is established, and it could act as a wound parasite. But in the pear it fails to develop further and kill the tree and seems to have very little effect upon the tree other than at the point where the lesion occurs. In this experiment no cases of pear trees clearly showing abnormal yellowing or wilting of the top were observed. Of the hundred or more trees in each lot an occasional tree died, but these were usually the small inferior ones, badly crowded; there was no certainty that death was due to *Armillaria*, although the fungus was sometimes found in the roots. It may enter any dead root. In contrast, when the susceptible Persian walnut and peach are entered no such reaction occurs. The fungus on reaching the cambium, or before, spreads rapidly, usually girdling the root and eventually causing death to the top.

The black walnut represents a species exhibiting considerable resistance in the field and has long been used as a resistant stock. Seed gathered from an isolated tree was planted in inoculated soil and the seedlings were dug from time to time. Lesions appearing on the roots were sectioned, and the penetration of the rhizomorph was studied in 14 separate infections where the fungus had penetrated through the root periderm. Of this number 12 had been checked and walled off by cork. The remaining 2 infections became deep and, at the time collected, had not been walled off by cork, although there was some indication in 1 that the spread had been arrested. In the latter, at about the usual position where cork formed in the others, there was found a line of cells the contents of which took the stain deeply and showed evidence of disintegration. While a high percentage of the infections in the seedlings examined had been checked at the first stage of infection, i. e., after the act of penetration, it does not mean that all are stopped at this stage, for many of the seedlings that were left in the inoculated soil eventually died and, on digging, were found to have their roots thoroughly permeated by the internal rhizomorphs of the fungus.

The essential features concerned with the penetration of the rhizomorph into the black walnut root vary but little from the manner in which the resistant pear root is invaded. In plate 10, *A*, is illustrated the very early checking of the penetrating rhizomorph and the subsequent formation of cork walling off the area. In this instance the rhizomorph penetrated only through the cork and was then stopped. The influence of the rhizomorph has extended a few cells beyond, as evidenced by the darker staining. Plate 10, *B*, illustrates another instance in which the rhizomorph has been checked and the affected area walled off by cork, but in this case a branch rhizomorph (*d*) is penetrating the secondary cork layer and is doing so in much the same manner as that illustrated in the pear (pls. 6, *A*, and 7, *A*). The rhizomorph is not entirely through the cork, but the cells in the tissue under it are plasmolized and darkly

stained, indicating the effect of some diffusible substance from the rhizomorph. The two lesions illustrated above are typical of those examined. In some the rhizomorph penetrated deeper and invaded more of the bark, but in only one instance did it appear that the infection was spreading. It must be admitted in this connection that only the smaller lesions were examined. In the larger lesions the fungus was undoubtedly making headway, otherwise some of the seedlings would not have been eventually killed. The essential difference between the infection of the susceptible Persian walnut root and the resistant black is that very few infections fail to become established in the former or become checked and corked out, whereas in the latter it is the rule rather than the exception that the rhizomorph is held in check and walled off by cork. Of the 14 infections comprising the study on the black walnut root in no case did the fungus enter at a branch root or at a lenticel. In two lesions the rhizomorph had penetrated the cork close to a small branch root, but there was no relation between the ruptured tissue around the root and the entrance of the fungus.

In the field the myrobalan is intermediate in resistance between the pear and peach. Pot experiments indicate that it has considerable resistance. Of 45 trees planted in pots only a very few actually died from *Armillaria*. Examination of the roots at various times after planting indicated that infection had occurred in practically all cases examined, and not only in one place on the root but usually in several. In one carefully examined root system 21 separate infections were noted. These surface lesions varied greatly in extent, ranging from as small as one fourth inch in diameter up to the entire circumference of a branch root. The tree was not dead and did not appear to be suffering greatly from the numerous disease lesions in the root. A more careful examination of the lesions indicated that the fungus was extending only very slowly in them, in some probably not at all. The borders of the lesions consisted of tissue which had turned a bright red and was extremely hard and brittle. It is possible that this layer, on account of its hardness, is in part responsible for the reduced activity of the fungus rhizomorph in these tissues, but more fundamental reactions must occur in the host before this layer is produced. The red zone is sometimes surrounded by cork but more often not. In the myrobalan the extent of the fungus in the woody cylinder of the root is often equal to that in the cortex. This differs from a susceptible host where the growth in the cortex and cambium is normally much more rapid than that in the wood.

CYTOLOGICAL ASPECTS OF INFECTION

Destruction of Cork

The studies thus far have given a general account of the histological features concerned with penetration and infection by the rhizomorph. More detail of some of the cellular phenomena will now be presented.

The dense mass of active hyphae composing the invading branch presses against the cortex of the root. At first, so far as staining reactions indicate, it has very little chemical effect upon the walls

of the cork layer. As the pressure continues, however, the suberized walls that are in direct contact with the active rhizomorph tip seem to disappear as if acted upon by some dissolving force. They undergo little, if any, swelling, and this only in the very latest stage before their complete disappearance. There is no general breakdown of the cork tissue for any distance away from the rhizomorph. The latter acts on and destroys only those cells with which it comes in contact and against which it exerts pressure. The rhizomorph may grow laterally in the cork and split it apart longitudinally (pl. 5, *B*) after once penetrating into it, but many of the cells that originally occupied the area where the rhizomorph has actually pushed through have disappeared and no remnants of them remain. Neither are they engulfed and then destroyed. If mechanical pressure alone were responsible for penetration and no solvent action took place, one would expect to find the cork cells heaped up or folded back around the point of entrance. This has never been observed to an extent sufficient to account for all the cells originally present. As the cork under the invading branch of the rhizomorph is gradually acted upon and disappears, the latter pushes through into the cells below.

Effect of the Rhizomorph on the Parenchyma Before Invasion

In certain of the hosts examined while the rhizomorph is in the process of entry, but before it has reached below the cork, certain reactions have taken place in the parenchyma directly under the penetrating rhizomorph which indicate that the influence of the rhizomorph is felt in the tissue below it. When the rhizomorph has penetrated into the second or third layer of the cork covering of the parsnip the parenchyma cells immediately under the rhizomorph tip take the red stain very deeply, when Flemming's triple is used (pl. 2, *B*, *a*). The cell contents stain somewhat deeper than normally, but the most noticeable effect is in the cell walls and the nuclei which stain much deeper than normally and indicate some influence of the rhizomorph. The walnut root often shows a very different reaction, from that described above, when the rhizomorph tip is still two or three cell layers away. The affected walls of the parenchyma directly under the cork, instead of taking an abnormal deep red as was the case in the parsnip, fail to take any stain at all and remain a light brown similar to that before any treatment. The cell contents are somewhat plasomolyzed and take the stain most deeply, becoming almost black in color. They are in striking contrast to normal cells with deep-red walls and lighter red contents. The fact that the cork cells in immediate contact with the advancing rhizomorph tip show so little disturbance possibly indicates their extreme resistance to fungal action in comparison with the cells just under the cork layer. There would seem to be little doubt that some substance of a toxic nature is given off by the invading rhizomorph tip which causes the apparent rapid death and chemical change of the cells below the cork layer. Yet, so far as the stains employed in this work indicate, this substance has comparatively little effect on the cork cells through which it must pass and where it would supposedly be more concentrated.

The lateral spread of this material is plainly more rapid in the tissue immediately under the cork than its depth of penetration into the tissue below. In plate 5, *B*, (walnut root) the extent of the disorganization is plainly visible. The disorganized cells extend laterally for five or six cell lengths beyond the ends of the penetrating branch, while at the center of this branch the downward extent is only three or four cells in their short axis. Other cases in the walnut were even more pronounced than this one. No evidence was found that toxic action to the host occurs under an attached rhizomorph which is not preparing to enter.

Effect of the Advancing Rhizomorph on the Tissue Surrounding It

After the rhizomorph passes the cork layer, it enters dead disorganized tissue and never penetrates ahead of the dead cells. The nature of this killing action has not been investigated. The distance to which it may extend beyond the rhizomorph tip varies with the host and type of tissue. The parsnip presents an exceptional case in that there is a network of large intercellular spaces to be found in the fleshy cortical tissue. The toxic action has been observed to extend as much as 5.5 mm beyond the invading rhizomorph tip. It appears that the toxic material, whatever its nature may be, follows the intercellular spaces in the parsnip instead of passing directly through each cell to the adjoining one as appears to be the case in woody tissues. In the latter the action may extend only a few cells away from the rhizomorph.

The potato tuber presents a type of tissue upon which the fungus is very active. There is a marked killing and browning of the tissue around the rhizomorph tip. This varies in extent but often involves cells several cell widths distant. Kusano (*25*) indicates that the cell sap is the part which becomes brown. Observed macroscopically this would appear to be true, but the investigations here reported do not support such a view. The observed browning is due to the formation of a granular mass which first forms in the small intercellular spaces where the cells meet. It is next observed to occur along the inside of the cell wall, where it at first forces the plasma membrane away. Later the membrane disappears, and the brown material completely fills the cell cavity and often becomes less dense. It has much the same staining properties as the cell wall, namely, a lack of affinity for any stain. These facts suggest that this material may be a decomposition product of the cell wall. The protoplasts of the less affected cells take the stain normally but when stained are severely plasmolyzed. This is in contrast to the surrounding normal tissue. The disappearance of starch from the affected area appears to follow no definite rule. It is sometimes removed from the cells at the time of appearance of the brown material. Other times it remains until the individual side hyphae have penetrated into the area. In the very late stages of decomposition and just before their total destruction, the cells immediately surrounding the rhizomorph collapse completely. Their walls fold inward and are pressed closely together by the mechanical force of the advancing rhizomorph. The folded remnants of the protoplast lie between these swollen walls of indefinite outline. The mass assumes a very dark stain

and then disappears entirely as the fungus rhizomorph grows against and into it.

While the growth of the fungus in the carrot is the same in its essential features as in the potato, the extent of diseased tissue away from the penetrating rhizomorph appears to be less and ordinarily extends but a few cells, perhaps indicating that the fungus is less parasitic on the carrot than on the potato. Gross observations lend some support to this belief. Once the potato has been penetrated, the fungus usually spreads rapidly and in all directions. In the carrot the spread may not be so extensive, as illustrated in plate 4, *B*, where the growth is slow and upon close examination shows the rhizomorph to be following small cavities or pockets in the central cylinder. The occurrence of these cavities is quite constant in the carrot but has never been observed in other hosts. On sectioning they appear as hollow spaces where the tissue has collapsed and drawn to one side. The relation of the cavity to the rhizomorph has not been fully determined. It appears always to connect at some point with the rhizomorph, but the latter does not ordinarily extend into the bulk of the pocket as might be expected if products from the fungus caused the collapse of the tissue.

The most noticeable effect of the fungus on the susceptible Persian walnut root is the rapid and complete browning of the tissue surrounding the rhizomorph. The cell walls are but little, if at all, swollen. They are definitely browned and have no affinity for any of the stains employed. No granular material collects along the walls as happens in the potato tuber. The original cell contents in the walnut root usually turn brown along with the wall or soon after and, like the wall, do not take the stain. Some of the cells fill with a very dense and darkly staining material, which, at least after being stained, is badly plasmolyzed (pl. 5, *A*, *b*). The formation of this substance inside the cell, along with the browning of the wall, is usually the first indication that the influence from the rhizomorph is felt below the cork.

A cell reaction, probably similar in effect to that described above when the walnut is invaded, is to be found in the myrobalan root. It is confined principally to that zone of red tissue that, in the myrobalan, usually borders a lesion produced by an invading or advancing rhizomorph, such as was described above under histology of infection. The occurrence and disappearance of the red zone may be followed best by examining unstained freehand sections cut through the border of the lesion, including the healthy tissue above and the disintegrated tissue close to the rhizomorph below. In such a section the live, healthy tissue entirely outside the lesion appears normal. The first indication of disturbance at the outer margin of the affected area is a slight yellowing of the cell walls in a region only a few cells in width. The contents of these cells, if at all changed, become less dense than normal. Then occurs a narrow zone in which the lumina of the cells are beginning to fill with a granular, colorless material, not dense at first but soon becoming so, and with this the red color develops, becoming more intense with the increase in density. This red zone often involves a region of considerable width. The red stain is entirely in the dense deposit filling the cell, which may normally occupy the entire lumen but under some conditions

shows plasmolysis. The walls remain yellow as at first or darken slightly but never become red like the cell contents. The red fades out as the rhizomorph is approached, and the tissue becomes a yellow-reddish brown, the cell contents becoming less dense and finally disappearing and the whole mass showing signs of disintegration. The regions above described may vary appreciably in extent and at times may not be so clearly defined as indicated, but the red zone appears never to be missing. When staining with Flemming's triple, a somewhat different picture is presented. The dense cell contents, whether colored or uncolored, shrink into a small mass at the center of the cell, staining deeply. The walls in this region do not take the stain and remain a light brown or yellow. The zone close to the rhizomorph assumes a rather deep-reddish stain characteristic of disorganizing tissue.

The red zone is not confined to the cortex tissue, but the same material may be deposited in the cells of the wood as well, although here the intensity of the red is sometimes lessened and more irregular in extent. When tested for lignin with Maule's permanganate test (Morrow (*29*)), the walls in the red zone and below take on an amber color while the normal walls above stain a very deep pink, indicating the delignification of the cell wall by the action of the fungus.

The effect of parasitism on the above-described roots differs in at least one respect from that on the resistant French pear. This difference manifests itself in the lack of visible effect upon the cell walls of the cortex of the pear root when invasion occurs. They show none of the browning or yellowing and have no affinity for stains which is so characteristic of affected walls in the other hosts studied and which was one of the first observable symptoms of parasitism in the Persian walnut, always preceding the growth of the rhizomorph into the tissue. That region of tissue in other woody hosts with brown walls and deep-staining granular contents, which usually precedes the narrow zone of more completely disorganized and deep-staining cell material in direct contact with the rhizomorph, appears to be lacking in the cortical tissue of the pear, and only the disorganized zone extending but a few cell widths away from the invading rhizomorph is present. In the young pear roots examined this contrast was quite evident.

Wound Gum

To all appearances the red, deep-staining substance which is deposited in the cells of the zone of affected tissue in the myrobalan is similar to that occurring in the walnut and might properly be termed "wound gum." Its formation and location with respect to the diseased tissue closely resembles the wound gum as described by White (*44*) when *Fomes applanatus* is acting parasitically upon various forest trees. He describes it in the beech as causing a band of dark-colored material which creeps forward as the fungus advances into the wood. It forms only in the newly attacked cells and disappears as the fungus advances, leaving no trace behind. The gum is described by others but there is some disagreement as to its origin. Tschirch (*39*) believes it to be a secretion of the living protoplasm bordering a wounded area. Münch (*30*) maintains that it is an oxidation product of the cell contents forming after their

death. White (*44*) finds tyloses with the wound gum and considers that they can only be produced from living tissue and consequently "if wound gum is not a secretion it follows very closely on the death of the producing cell."

In these investigations the process was excellently demonstrated at the time of the very early stages of rhizomorph penetration into the Persian walnut root. As described before, the formation of this deep-staining material, which may now be called wound gum, was one of the first indications that the invading rhizomorph was acting below the cork layer. The browning of the cell wall and the formation of the wound gum appear to occur almost simultaneously; however, the wall sometimes browns without the formation of the gum. If the browning of the wall is indicative of the death of the cell and a normal-staining wall indicative of a healthy cell, then it is necessary to assume that the formation of the wound gum occurs with the death of the cell or shortly after. This would not support the view that it is a secretion of the living cell. If, on the other hand, the wall shows signs of browning before the protoplasm is dead, it may possibly mean that the wound gum is a secretion of the living cell. The former view seems rather more probable and is given some support in the myrobalan where at times there occurs a narrow zone of cells with yellowed walls between the normal tissue and the wound-gum area, thus indicating some disturbance, possibly death, ahead of the wound gum.

Staining Reactions Before Complete Destruction

In the Persian walnut and myrobalan as in the potato, the narrow zone of tissue immediately surrounding the rhizomorph, when in the last stages before complete destruction, assumes a very deep red color with Flemming's triple stain and a rather dark brilliant green with Pianeze's. The colors, no doubt, represent the complete chemical change which occurs in the tissue as decay and distintegration proceed.

Browning of the Woody Cylinder

When an examination is made of the attacked woody tissues of the above hosts, the differences between the pear and the susceptible hosts are not at all striking. A common feature macroscopically observable in all the woody hosts, when *Armillaria* has formed a deep lesion and invaded the tissue below the cambium, is the browning of the woody cylinder, more noticeable on the side where the fungus is active, but sometimes penetrating deep into the cylinder in the vicinity of the lesion and extending up and down the wood a short distance. It affects the wood much deeper than any of the branching hyphae appear to penetrate. The browning is due to the occurrence in the vessels of the wood of a gummy-appearing material varying in color from a light-yellowish brown to a rather dark-reddish brown. It fills only a part of the wood vessels, the color of the wood becoming darker as more vessels are involved. It is believed that this is a different form of wound gum from that occurring within the cell. It may be a secretion into the vessels from these affected cells or a product from their walls. At first it

is a light yellow and has much the same color as the cell wall in the region where formed, but it darkens with age. It does not ordinarily possess the granular appearance so noticeable in the red wound gum inside the cells of the myrobalan root. No destruction of the tracheal wall was observed at this stage of infection in any of the hosts examined.

FORMATION OF CORK

Cork formation around the infected area is not entirely limited to resistant hosts but was observed in all hosts examined, with the exception of the potato. Its occurrence is not constant in either susceptible or resistant roots, although when a lesion is formed and fails to spread a cork layer is often subsequently developed.

As first observed, a few cells in the parenchyma of the cortex several cells distant from the rhizomorph divide. A phellogen soon surrounds the affected area, and as this becomes active cork is formed. Since the stains employed do not clearly differentiate a suberin wall, it was impossible to be certain when the developing cells of this area first showed a suberin reaction. Freehand sections from many of these hosts stained with Sudan III took the typical deep-orange stain for suberin, when only a few layers were produced by the phellogen. The reaction may occur much earlier, but the proof is lacking. In a susceptible walnut this layer was never observed to attain a thickness of more than 3 or 4 cells, when surrounding an active rhizomorph. At about this stage it showed signs of disorganization, and further division of cells ceased. It is unknown whether or not suberin was deposited in these walls at this time.

It can be seen in the pear in plates 6, *A*; 7, *A* and *B*; and 8, *A*; and in the black walnut in plate 10, *A* and *B*, that cork forms and walls off the disorganized tissue around the rhizomorph. Plate 7, *A*, is an enlargement in the cork area. Although not illustrated, small lesions were found in the pear where no formation of cork had taken place and from appearances the rhizomorph was no longer active. In such cases, the dead, browned tissue may stop at as definite a line as if a cork layer were present. The dead cell is brown, but the one next to it may be perfectly normal in appearance. Instances of this nature lead one to question whether the cork is primarily a mechanical barrier preventing the spread of the fungus or merely forms after some other factor has checked the growth of the fungus. The latter seems more probable.

GUM CAVITIES

A discussion of the effect of *Armillaria mellea* upon deciduous fruit trees would not be complete without further mention of the gum cavities which occur in connection with this disease. So far as the author has been able to determine, no mention of this phenomenon has been made by anyone describing the effect of this disease on fruit trees. Of the many hosts examined it has never been found on any except those belonging to the genus *Prunus*. (The roots of *Citrus* have not been examined by the author.) Hartig (*16*), however, probably refers to a somewhat similar phenomenon in pine roots when he describes the abnormal resin and turpentine canals occur-

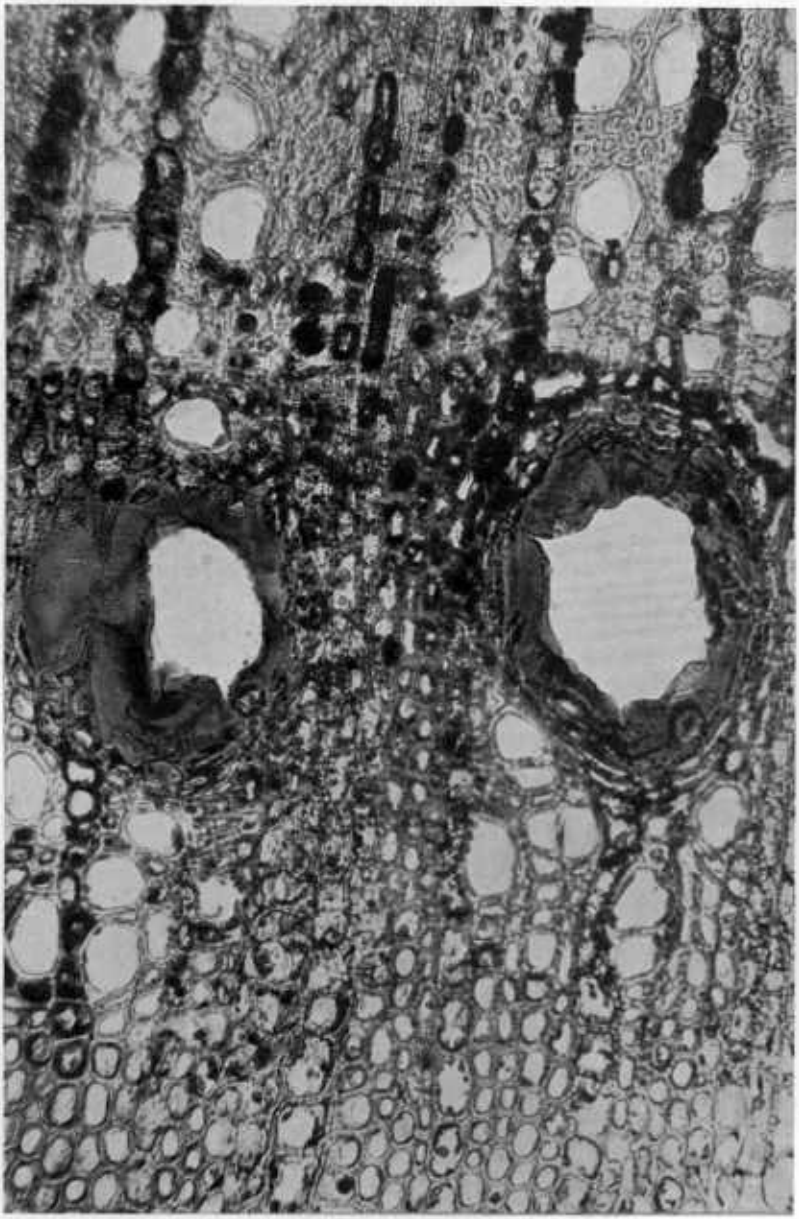
ring in the region of the cambium. He claims that resinous materials flow into this region from the medullary rays, causing large abnormal canals in the "wood ring formed during the year of sickness."

In species of *Prunus* it has been a common observation that large quantities of gummy material exude from the base of many fruit trees when attacked by *Armillaria*. It often infiltrates into the surrounding soil where it dries and hardens to form a stony mass.

The formation of gum in species of *Prunus* has been investigated by many authors. A thorough discussion of its formation would be beyond the scope of this study. Butler (*9*), working with *Prunus* and *Citrus*, found its formation induced by "all manner of traumatisms" and that many chemical agents as well as various fungi were effective in producing it. He considers that the gum is due to the "hydrolysis of the walls of the embryonic wood cells", the action proceeding centripetally from the secondary lamella and finally reducing the whole cell to a mass of gum. Goldsworthy [3] credited bacteria with causing much of the gummosis in *Prunus*. It is thus seen that the production of gum may be due to a variety of causes, with the probability that organisms play an important role. There is no proof that the gum pockets here described are due entirely to the action of *Armillaria*. Bacteria might be present in conjunction with the fungus and produce the effect, but since other fungi cause gumming and since the phenomenon occurs almost constantly in connection with *Armillaria*, it is believed that the action is due to *Armillaria* alone.

Plate 11 represents typical gum cavities as observed in a prune tree attacked by *Armillaria*. The cavities most frequently occur in the layer of young wood cells just inside the cambium but are not limited to this region. At times they extend out into the phloem, apparently destroying the cambium. They are rarely confined to the phloem. If the root is small and completely girdled by the fungus, cavities may extend in a row entirely around the circumference of the root. Otherwise they extend as far as the fungus is operative. Occasionally two rows of cavities may form, one outside the other and rather close together. The medullary rays are seldom involved except as pressure may alter them. That pressure is devoloped in these cavities is evident from the manner in which the cells are compressed around the edges of the cavity; this suggests that while the tissue was young, gum was forced up from below into the area, and the cells were crowded apart and flattened tangentially around the gum column. At times very little solvent effect upon the cell walls is observable, but ordinarily some of the original cells are lost, probably contributing to the gum mass. Cavities are often found in which the cells surrounding them have inflated and extend into the cavity like tyloses into wood vessels. To explain this, it might be assumed that the pressure once developed later diminishes, allowing the still living cells around the sides to grow and expand into the cavity.

[3] GOLDSWORTHY, M. C. GUMMOSIS IN THE GENUS PRUNUS. (Doctorate thesis, Univ. Calif.)



Prune roots; gum cavities in young wood. × 263.

An examination of a longitudinal section of the affected tree trunk, including an area beginning with the healthy normal tissue above and extending down to the active rhizomorphs, would show somewhat the following: At the base of the section the rhizomorph is active in the cortex and cambium, sending out from its surface radiating hyphae which enter the outer layers of the wood as well as the cortex, act on and destroy it, and in so doing produce the gummy material, or produce substances which act on the young woody cells above, hydrolyzing their walls into this material, much as described by Hartig (*16*) in the case of the pines when turpentine or resinous material was produced. The gummy substances appear to follow more or less the vessels of the wood above the extending rhizomorph tip. The latter follows this gummy material that fills the cavities, destroying it or forcing it out as the rhizomorph proceeds upward. Many of the cells in this region are destroyed, indicating that the products of metabolism of the fungus are probably operative in destroying the young wood cells, similar to the chemical action described by Butler (*9*). During this destructive process the mass of gum is under pressure. Some of it is forced to the outside through the now disorganized cortex, and some is forced up into the young tissue around the cambial region, producing what has been observed before in cross section. As the fungus rhizomorph advances, this tissue is soon destroyed and the whole process gradually moves upward. This appears to be the most logical explanation, from the sectioning of many roots. The greatest distance which these cavities may extend up above the rhizomorph has received no critical attention, but they were observed to occur at a distance of 1 inch above the rhizomorph in a peach root.

GROWTH OF ARMILLARIA ON EXPRESSED SAP OF VARIOUS TREE ROOTS

In view of the results obtained in the histological and cytological investigations, indicating that resistance is a factor not concerned with any structural or morphological character of the host, a study was made of the fungus growth on the expressed sap of several tree roots, including both susceptible and resistant ones. The behavior of the fungus in attacking the resistant pear suggests that there may be some substance in the host which inhibits the growth of the parasite. If such an inhibiting substance is present in the cell sap and is of a stable nature not easily oxidizable or destroyed, it might be possible to test resistance by growing the fungus upon the expressed sap of the host.

A study of this nature was undertaken with the roots of several trees. The bark only was used, since the factor for resistance, if such be present, must be located in that portion of the root as well as in the wood. Roots to be tested were dug and protected from loss of water until such time as the sap could be expressed, when they were thoroughly washed and the bark peeled off and run through a meat grinder. The sap was then expressed from this ground material in a hydraulic plant press, using a pressure of 350 kilos per square centimeter. If any quantity of solid material was pressed out, the sap was centrifuged. As a means of sterilization the sap was

filtered through a candle. In the first experiment a Chamberland-Pasteur filter was used, but in subsequent work a Berkefeld V was employed and with it a modification of the aseptic filter apparatus as described by Smith (*37*). The filtered sap was tubed in 3 to 5 cc lots, and in experiments after the first was allowed to stand for 3 or 4 days when two streaks were made from each tube to determine if contamination had occurred. This precaution was probably unnecessary as very few instances of contamination were ever found. The sap was then inoculated with *Armillaria* usually with the brown surface crust which forms when the fungus is grown on prune-agar slants. Care was necessary that the colony remain afloat because the production of internal rhizomorphs from a submerged colony was never observed. When growth starts at the surface the rhizomorphs develop after varying periods of time and grow down into the medium.

In the first experiment the roots of oak, *Quercus agrifolia;* fig, *Ficus carica* var. *sylvestris;* peach, *Prunus persica;* and apricot, *Prunus armeniaca* were employed. The oak and fig are fairly resistant, whereas the peach and apricot are quite susceptible. In this experiment the ground material was frozen before expressing, a procedure not used in later work. Some of the tubes in each lot were heated to stop enzymatic action. The data are given in table 1. Unfiltered sap was also inoculated, but in most instances it failed to support growth. Because of the probability of contamination little weight could be placed on the results, and they are therefore not reported.

TABLE 1.—*Growth of Armillaria mellea on the expressed sap of oak, fig, peach, and apricot roots*

[Sap expressed Mar. 23, 1926]

Root	Sap treatment	Tubes	Tubes showing growth	Remarks
		Number	*Number*	
Oak	Filtered	5	4	Growth slow.
Do	Filtered and heated [a]	3	3	Do.
Fig	Filtered	5	5	Growth good.
Do	Filtered and heated [a]	4	4	Do.
Peach	Filtered	5	0	
Do	Filtered and heated [a]	4	0	
Apricot	Filtered	5	4	Growth less vigorous than on fig.
Do	Filtered and heated [a]	4	1	Do.

[a] Placed in boiling water for 15 minutes. The contents of the tube reached 97° C.

The most striking feature of these results is the fact that the fig and oak roots, which are decidedly resistant to *Armillaria*, showed very little inhibiting effect on fungus growth in the expressed sap, while the peach sap prevented growth entirely, yet the peach is one of the most susceptible of hosts. In all lots the heating of the sap almost to the boiling point had little significant effect upon the growth of the fungus. In the peach the growth-inhibiting substance is evidently thermostable.

Although the foregoing results gave little reason for believing that expressed sap might be a means of testing resistance, it was decided to make further trials, and in so doing to select a susceptible and a resistant species belonging to the same genus, in order that their dissimilarities might be less. The northern California black walnut, *Juglans hindsii* (resistant), and the Persian walnut, *J. regia* (susceptible), offered such a combination. The studies were carried on over a period of more than a year to determine if any seasonal change in the tree may affect the growth of the fungus on the expressed sap. A 3-year-old northern California black walnut (designated no. 1) growing in an "*Armillaria* spot" in an orchard was used in the experiment. Roots were removed from it at various intervals during a period of 17 months. Northern California black walnuts no. 2 and no. 3 were composite lots of roots of seedling trees grown in large pots for 2 years. The bark from the roots of several trees was composited and the sap expressed.

Persian walnut no. 1 was a chance seedling 4 or 5 years old. Persian walnut no. 2 was an old seedling planted as a border tree along the highway several years ago. Portions of the sap expressed on October 1, 1926, of both black walnut no. 1 and Persian walnut no. 2 were sterilized by adding a few drops of chloroform, shaking for a few minutes and evaporating off the chloroform by bubbling sterile air through it. This acted as a partial check against the possible removal in filtering of substances which might be essential as growth-inhibiting agents. Filtered, chloroformed saps were used as checks on the method. The chloroform treatment was found to have no influence on the growth of the fungus different from that obtained when the filter alone was used. It was therefore not used in later work. The data are presented in table 2. Growth failed on the sap from black walnut no. 1 in all trials except the first and the last. In the first, growth was good, but in the last trial only 2 out of 5 tubes supported growth and then only poorly. It is not believed that these differences are due to seasonal changes in the tree, for on July 20, 1926, the fungus growth on the sap was good but failed entirely on the same date 1 year later. That this factor of growth inhibition in the expressed sap is not constant in all black walnut trees is evident when the data relating to the black walnut seedlings no. 2 and no. 3 are compared with those of black walnut no. 1. In the former, growth was good, even better than on the supposedly susceptible Persian walnut, while in the latter it was almost a failure. Growth always occurred on the sap of the Persian walnut root, although not exceptionally good at all times. That the inhibiting factor in black walnut no. 1 is thermostable is evident from the results obtained with the sap expressed on July 20, 1927, which was heated in boiling water for a period of 15 minutes.

TABLE 2.—*Growth of Armillaria mellea on the expressed sap of northern California black and Persian walnut roots*

Root	Date expressed	Sap treatment	Tubes	Tubes showing growth	Remarks
			Number	*Number*	
Black walnut no. 1	July 20, 1926	Filtered	5	5	Growth fair.
Do	Oct. 1, 1926	do	8	0	
Do	do	Centrifuged, sterilized with chloroform.	2	0	
Do	do	Filtered, treated with chloroform.	3	0	
Do	Apr. 6, 1927	Filtered	8	0	[a]
Do	July 20, 1927	do	11	0	
		Filtered and heated	5	0	
Do	Dec. 22, 1927	Filtered	5	2	Growth poor.
Black walnut seedlings no. 2.	June 3, 1927	do	19	18	Growth very good.
		Filtered through Seitz filter	7	7	Do.
Black walnut seedlings no. 3.	June 2, 1927	Filtered	9	9	Growth good, but less than no. 2.
Persian walnut no. 1.	July 20, 1926	do	5	5	Growth poor.
Persian walnut no. 2.	Oct. 1, 1926	do	9	9	Growth fair.
		Centrifuged, sterilized with chloroform.	3	3	Do.
		Filtered, treated with chloroform.	3	3	Do.
Do	Mar. 26, 1927	Filtered	6	5	Growth good.

[a] One month after first inoculation failed, one half of the tubes were heated in boiling water for 15 minutes and all reinoculated. Fungus again failed to grow in any tube.

Table 3 presents the results of culturing on the diluted sap of black walnut no. 1, using both prune decoction, which supports the growth of *Armillaria* very well, and distilled water as the diluting substances. Certain amounts of sap when added to prune medium are evidently invigorating to the growth of this fungus, and even when mixed in the high proportion of 1 part of sap to 1 of decoction growth is better than on the prune decoction alone, yet the expressed sap alone fails to support the growth of the fungus. When water is used as the diluting substance the results show that growth takes place and is best when the sap is diluted 1 to 1.

TABLE 3.—*Effect of dilution of the expressed sap from roots of resistant black walnut no. 1 on growth of Armillaria mellea*

Date expressed	Dilution no.	Volumes of—			Tubes	Growth
		Sap	Diluting substance			
			Quantity	Material		
		Cc	*Cc*		*Number*	
July 20, 1927	1	0.0	5.0	Prune decoction	5	Good, but not extremely vigorous.
	2	0.1	5.0	do	5	More vigorous than in dilution no. 1.
	3	1.0	4.0	do	5	Most vigorous of all lots with prune decoction.
	4	2.5	2.5	do	5	Vigorous, about like dilution no. 2.
	5	4.0	1.0	do	5	In 2 tubes only, and this poor.
	6	5.0	0.0	do	11	None.
Dec. 22, 1927	1	1	4	Water	5	Weak; similar to that in weak prune decoction.
	2	2	2	do	5	Most vigorous of water-dilution series.
	3	2	1	do	5	Slightly less than in dilution no. 2.
	4	3	0	do	5	In 2 tubes only, and this poor.

The results given in table 1 indicated that the expressed sap of the peach was inhibitive to the growth of the fungus. This was further tested with roots from the same tree, with roots from two other trees, and with a composite sample taken from a half dozen peach nursery trees. The results are given in table 4. In only two tubes did the fungus start and grow. Reinoculating after a period of 2 months showed that the growth-inhibiting substance was still present. Not only was growth inhibited but the inoculum was actually killed, for when removed and placed on prune agar, it failed to grow.

Table 4 also presents data regarding growth on the expressed sap of the roots of a composite sample of French pear seedlings. Growth was vigorous and rapid and differed little from that on the expressed sap of the susceptible Japanese pear (*Pyrus serotina*). On the sap expressed from myrobalan seedlings growth was positive but less vigorous than on the pear.

TABLE 4.—*Growth of Armillaria mellea on the expressed sap of peach, French pear, Japanese pear, and myrobalan roots*

Root	Date expressed	Sap treatment	Tubes	Tubes showing growth	Remarks
			Number	*Number*	
Peach no. 1	Feb. 1, 1927	Filtered	8	1	Inoculum died in others.
	Apr. 1, 1927	Reinoculated	7	0	
Peach no. 2	Feb. 1, 1927	Filtered	5	0	Inoculum died.
	Apr. 1, 1927	Reinoculated	4	0	
Peach (nursery trees)	Mar. 26, 1927	Filtered	6	1	
French pear seedlings	Apr. 26, 1927	do	9	9	Vigorous and rapid growth.
Japanese pear	Apr. 25, 1927	do	9	8	Do.
Myrobalan seedlings	May 5, 1927	do	14	11	Not as rapid growth as in pear.

It is evident from these data that growth of the fungus on expressed sap of the bark of tree roots is not indicative of the susceptibility of the root to *Armillaria*, nor is the failure of growth indicative of resistance. The only instance of positive correlation was with black walnut no. 1; this varied and was not constant.

DISCUSSION

The modes of infection employed by root parasites have received little attention from investigators. A method of entry appearing to resemble most closely that of *Armillaria* was described by Peltier and Samson (*33*) in the case of *Ozonium omnivorum*. They state that by mechanical force hyphal wedges from the fungus strands on the root surface push in between the cork cells and finally engulf them in the fungus mass. The cells soon collapse but are not destroyed. Penetration occurs most commonly at a lenticel but may be directly through the cork. Another instance of root entry was described by Conant (*11*) who believed that the hyphae of *Thielavia basicola* in very young tobacco roots may mass together at times, and so weaken the suberized wall by enzymatic action that they are able to "surge" through. This, however, is not the usual method of entry. No single hyphae of this fungus were ever observed to penetrate a suberized wall.

It is stated by Appel (*1*) that species of *Phytophthora* and *Fusarium* penetrate a thin cork layer but not a thick one. Lutman (*27*) thought that the thickness of the skin may be partly responsible for resistance to potato scab. It might be argued in these cases that mechanical pressure is involved, otherwise the thickness of the cork would be relatively unimportant. Tisdale (*38*) in a study of flax wilt states that *Fusarium lini*, the cause of the disease, can penetrate the epidermis of the young roots; but when wound cork is developed around an infection inside the root, the fungus does not penetrate through it. He thinks it possible, however, that some reaction of the host protoplasm may weaken the fungus at the same time. Fawcett (*13*) states that *Pythiacystis citrophthora* is able to invade citrus roots through uninjured cork layer, but only if abundant moisture is present with favorable soil temperatures over a sufficiently long period of time.

From the fragmentary evidence presented in the literature it might be concluded that root parasites do not commonly enter through the uninjured and healthy cork layer, but occasionally, when this does occur, they may enter either by means of mechanical force or chemical dissolution of the cork wall. *Armillaria* seems to present a more definite case of entry through the thick cork layer of a comparatively old root than any other fungus hitherto reported. According to the results of this investigation, the usual method by which *Armillaria mellea* gains entry into a root is by penetration of a rhizomorph branch directly through the sound cork layer into the tissue below. Of the numerous infections examined, no instance of definite entry through the ruptured tissue around a newly formed branch root where it emerges from the parent root, as suggested by Zeller (*46*), and by Rayner (*35*), was observed. Occasionally entrance is gained through a lenticel, and in such instances the method is similar to that through the cork layer. Under no circumstances was there evidence of a splitting of the cork cells at their middle lamellae. The rhizomorph branch enters as a unit, apparently employing both mechanical and chemical means in its penetration of the root periderm. It appears to be almost unique in its method of forcing through the suberized walls of the cork layer, as a single unit, the comparatively great bulk contained in a rhizomorph branch.

Kusano (*25*) and Day (*12*) concluded that this fungus had the power of destroying suberized tissue when the rhizomorph branch penetrated, and the evidence presented in this study gives further support to the same view. If the cork is thus actually dissolved it would seem necessary that some enzyme be associated with the process. A search of the literature failed to reveal that any specific enzyme produced by micro-organisms and capable of attacking suberin has ever been demonstrated. On this point Waksman (*42*) states "so far as our present information is concerned, cork and cutinized lamellae are not acted upon to any extent by micro-organisms." The action upon the cortex cells by *Armillaria* is not extensive and might fall within Wakman's "not acted upon to any extent," but where a group of cells is actually dissolved, as occurs in this case, the process could hardly be placed in that class. Since

the normal action by micro-organisms is enzymatic, it would seem that some enzyme may be involved in this case.

The evidence presented establishes beyond much doubt that *Armillaria mellea* can readily enter the sound, healthy roots or tubers of both susceptible and resistant plants. The apparent ease with which the fungus enters through the cork layer of all the hosts investigated makes it seem doubtful whether resistance to this fungus in any plant can be due entirely to the prevention of entrance. It would seem more logical that the second act of the fungus, the establishment of parasitic growth in the host, is the feature that decides whether this fungus is or is not to become a parasite. If such is the case, wounds would not play such an important part in this disease as other authors have assumed. They may, however, have a secondary effect in facilitating the establishment of the fungus by affording it saprophytic nourishment.

Conant (*11*) in his study of *Thielavia* root rot of tobacco decided that fungal invasion stimulated phellogen development in advance of the fungus and was of the opinion that the layer of cork was effective in walling off the fungus. Butler (*8*) discusses the defensive action of what he terms reactionary cork or that developed as the result of damage by invading parasitic fungi. He cites shot hole and pear and apple scab as examples of diseases where reactionary cork forms and prevents further spread of the fungus. He states that it often happens in these diseases if the fungus is growing vigorously that the plant is unable to form a continuous corky layer and consequently unable to prevent penetration into the tissue below at all points. A new layer of cork may then form and the process be repeated several times. He apparently considers, in the cases mentioned, that the formation of the reactionary cork is primary in function in the walling-off of the parasite and is not in any way secondary. In *Armillaria* root rot a phellogen is often produced far in advance of the penetrating rhizomorph and is especially noticeable in the resistant roots. It might be assumed that it is walling off the fungus and preventing its spread. The rhizomorph readily penetrates cork tissue, however, and it would therefore be expected that little good would be accomplished by the secondary periderm. That this is the case is proved by the instances found in which the rhizomorph had apparently penetrated directly through the second cork layer. It would thus appear that in the pear the formation of the wound cork is not a factor responsible for the resistance exhibited by this host. Other instances noted, in which the action of the rhizomorph had apparently ceased without the development of cork around it, lends more weight to the belief that cork formation is not a factor of resistance.

Studies on the growth of the fungus on the expressed sap show no significant correlation between inhibition of growth and resistance of the host. Of the resistant species, only in the root of a single black walnut was growth inhibited on the expressed sap, and even in this root, when the sap was added to prune decoction upon which the fungus ordinarily grows well, when not too concentrated, it actually produced more vigorous growth of the fungus. It, however, remains an unproved possibility in this particular instance that the osmotic

pressure of the expressed sap of this black walnut may be sufficient to inhibit fungus growth. In case this factor is responible it is not a constant one for the expressed sap of all resistant roots. Hawkins (*19*) is of the opinion that parasitic fungi will grow on solutions with a much higher osmotic pressure than the expressed sap of their hosts. The failure of *Armillaria* to grow on the expressed sap from peach roots may possibly be due to the products developed in the enzymic destruction of the glucoside amygdalin. In tubes containing expressed sap from peach roots the odor of hydrocyanic acid was always very distinct.

Several authors including Butler (*7*), Vavilov (*41*), Howitt (*23*), and Walker (*43*) have given reviews of the literature on disease resistance in plants, pointing out the principal factors responsible for resistance. In the present work on *Armillaria mellea* the histological evidence does not demonstrate that structural differences of the hosts are concerned. The primary and secondary cork layer proved to be an ineffective barrier. In fact, there seems to be no proof that resistance is of a mechanical or morphological nature; or as Vavilov (*41*) classifies resistance, it is not a case of "mechanical or passive immunity." If one follows Vavilov it must then fall in his other class, "physiological or active immunity." Certain factors are obviously unimportant in this class.

Positive or negative chemotropism in the sense suggested by Massee (*28*) would not explain the entrance into the resistant host with subsequent inhibition of the parasitic action. If chemotropism was a factor initiating penetration, it would not be expected to hinder growth after invasion had once occurred.

The acidity of the cell sap as a factor concerning resistance is a much debated question, but it is doubtful if it would be at all concerned in *Armillaria* resistance. Wolpert (*45*) has shown that an acidity corresponding to a pH value of from 2.0 to 2.9, varying with the media used, was necessary to inhibit the growth of *Armillaria* in artificial culture. It is doubtful if the acidity of the cell sap would ever approach this figure.

The fungus penetrates through the cortex of the resistant pear root and into the wood below the cambium, at times killing and destroying some wood. The fungus and its parasitic action finally come to a standstill, cork forms in the cortex walling off injured tissue. The cambium around the edges of the lesion produces new wood and cortex tissue, which in time heals over the wound. These facts lead one to believe that there is some antagonistic factor concerned with the tissue of the root which finally overcomes the parasitic action of the fungus and prevents its further spread. Furthermore, this factor appears to be limited to the active, healthy tissue of the root as evidenced by trials in which 2- to 3-inch root cuttings of the pear were placed in test tubes containing a few cubic centimeters of water and inoculated on the upper cut end. The fungus grew down the cambium quite readily. Such roots can hardly be considered in an active, healthy state; neither can they be considered as dead. From these trials one can at least conclude that there is nothing in morbid tissue of a pear root which inhibits growth. This observation, together with the fact that the fungus grows very

readily on the expressed pear sap, gives strong evidence that resistance in the pear must be concerned with the healthy growing tissue.

Klotz (*24*) suggests that resistance in *Citrus* to *Pythiacystis citrophthora* may be of the nature of a paralyzing or inhibiting effect upon the enzymes produced by the fungus by some substance present in the plant. If this is an explanation of resistance to *Armillaria*, it must be assumed that the paralyzing factor in the host tissue is not expressible with the cell sap or is in some way changed and rendered inactive by this procedure. Otherwise it could hardly be expected that *Armillaria* would grow so vigorously on the expressed sap, unless the enzymes of the fungus concerned with parasitism are different from those concerned with saprophytism.

While the manner in which the plant is attacked and overcomes the effect of the parasite might be thought of as something similar to antibody production in the animal system, there are no data to support such a belief.

From the evidence presented in this investigation one is led to believe that resistance is due to some antagonistic factor contained in living, healthy plant parts, which cannot be expressed with the cell sap, and is not present to any degree in morbid tissue.

SUMMARY

This paper presents the results of an investigation to determine the mode of entrance and subsequent development of *Armillaria mellea* in various susceptible and resistant roots and tubers. Observations on the growth of the fungus on expressed sap of various susceptible and resistant hosts are included. There exists a possibility that such studies might throw some light upon the nature of resistance.

Invasion of the root is accomplished by the penetration of a branch of the parent rhizomorph directly through the sound, healthy periderm of the host. The method is similar in the different hosts investigated, with no apparent difference between susceptible and resistant ones.

The branch penetrates as a unit and was never observed to send out single hyphae into the host ahead of it.

Penetration is partly by mechanical and partly by chemical means. There appears to be some destruction of the suberized walls as if they were acted upon by a suberin-dissolving enzyme.

Death of the cells always precedes the further advance of the rhizomorph into the tissue. In susceptible hosts the killing usually extends further away from the rhizomorph than in the resistant ones.

In susceptible roots, after entry has once been gained, the rhizomorphs grow rapidly and cause general destruction of the host tissue.

In resistant roots the fungus readily gains entrance but is unable to establish itself and ordinarily destroys but little of the affected root. The wounds thus formed soon cork out or heal over.

Wound gum was observed in the border of the lesions in some hosts. It was most noticeable in the walnut and myrobalan.

A secondary cork layer often forms in resistant hosts, walling off the wound made by the invasion of the fungus. Its significance

as a factor pertaining to resistance is doubtful, since the fungus readily breaks through such layers.

Gum cavities, which are of almost constant occurrence in species of *Prunus* affected by this fungus, are described and discussed.

The fungus grows well on the expressed sap of certain roots and not at all or only very poorly on others, but there seems to be little correlation between the inhibition of growth in this manner and the resistance of the living host.

Structural or morphological differences of the host probably exert little influence on resistance.

Resistance to *Armillaria mellea* appears to be of the nature of an antagonistic influence exerted upon the fungus by the host only when the latter is in an active, healthy state.

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